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CONTENTS

	PAGE
Field Studies on Nodulation and Growth of *Centrosema pubescens* Benth. By G. D. BOWEN	253
Specificity and Nitrogen Fixation in the Rhizobium Symbiosis of *Centrosema pubescens* Benth. By G. D. BOWEN	267
French Bean Variety and Spacing Trial. By G. W. J. AGNEW	283
Effect of Maturity and Tree Age on the Behaviour of Queensland Grown Delicious Apples Stored at 34–36°F. By C. D. STEVENSON	291
Blue Mould (*Peronospora tabacina* Adam) of Tobacco in North Queensland: Some Aspects of Chemical Control. By W. PONT	299
DDT for the Protection of Stored Potatoes. By A. W. S. MAY	329
Studies on Codling Moth Control in the Granite Belt. By A. W. S. MAY and M. BENGSTON	355
Strawberry Root-knot Nematode Investigations in Queensland. By R. C. COLBRAN	365
Mild Strain Protection as a Means of Reducing Losses from the Queensland Woodiness Virus in the Passion Vine. By J. H. SIMMONDS	371
Studies of the Coccoidea. 10. New Species of Diaspididae. By A. R. BRIMBLECOMBE	381
Freeze-drying of Starter Cultures. By W. F. SCHUBERT	409
A Biochemical Study of 141 Strains of Enterobacteriaceae Isolated from Tissues and Fluids of Animal Origin. By ELIZABETH TURNER	419
Some Aspects of Oestrus in Cattle, With Reference to Fertility on Artificial Insemination. 2. Crystallisation Patterns in Cervico-vaginal Mucus. By G. R. FALLON and J. M. CROFTS	431
Some Aspects of Oestrus in Cattle, with Reference to Fertility on Artificial Insemination. 3. Body Temperature and the Oestrous Cycle. By G. R. FALLON	439

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DECEMBER, 1959.

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FIELD STUDIES ON NODULATION AND GROWTH OF *CENTROSEMA PUBESCENS* BENTH.

By G. D. BOWEN, B.Sc.

(Formerly Plant Pathologist, Division of Plant Industry.)

SUMMARY.

Field growth and nodulation of Centrosema pubescens in northern and southern Queensland have been studied.

Plant growth has been considered in terms of yield, growth rates, net assimilation rates, and leaf area indices. The yields followed a sigmoid curve. Growth rate and net assimilation rate increased to a maximum at the time of stolon production. While the growth rate was maintained at this level until winter retardation and recession of growth, net assimilation rate continually declined following stolon production. Net assimilation rates in northern and southern Queensland were similar. Winter retardation of growth occurred when minimum screen temperatures were below 55 deg. F. and maximum screen temperatures below 78 deg. F.

A periodicity in nodulation was observed. Nodulation continued throughout growth. Following stolon formation nodulation occurred on stoloniferous roots, while there was a progressive elimination of older nodules on the tap-root system. There was no indication of a progressive elimination of smaller nodules. Nodulation and nodule senescence are considered to be governed by vegetative growth of the plant rather than other plant factors such as flower production.

Nitrogen analyses showed that only a small proportion of the plant nitrogen is contained in the nodules and only 4-20 per cent. in the underground parts.

Cutting to simulate heavy grazing resulted in a loss of two-thirds of the plant roots by weight and inactivation and sloughing of a major proportion of the nodules.

I. INTRODUCTION.

Centrosema pubescens Benth., a legume native to South America, has been used in many tropical and subtropical environments as a cover crop (Whyte, Nilsson-Leissner and Trumble 1953). In Queensland, however, it is becoming increasingly important as a legume component of tropical and subtropical pastures, particularly on well-drained coastal and sub-coastal soils in areas with an average annual rainfall greater than 45 in. Sloan (1957) recorded a doubling of carrying capacity in trials with a centro-para grass mixture compared with

para grass alone. Bowen (1959) indicated the specificity of this legume with respect to nodulation and also recorded the nitrogen-fixing efficiency of naturally occurring strains of Rhizobium on this legume. This paper deals with the course of nodulation with respect to plant growth in the field and the effect of management on these. Most reports to date on growth and nodulation of tropical legumes have been of a general nature only.

Although micro-environment and plant habit may be different in a pasture mixture than in a pure stand, the present investigations were carried out to form a basis for later work on the legume in association with grass.

II. FIELD STUDIES OF SEASONAL EFFECT ON PLANT GROWTH AND NODULATION.

In order to study nodulation with respect to plant development, plant growth studies were carried out at Ormiston (latitude 27 deg. S.) near Brisbane in southern Queensland and at Ayr (latitude 19·5 deg. S.) in North Queensland. The trial at the latter centre was made possible by the co-operation of the officers of the Ayr Regional Experiment Station. The detailed studies were supported by observations in other areas.

(1) Method.

At both sites inoculated seed was sown in rows 4 ft. apart in mid-November, 1956. The area available for the experiments was such that inclusion of uninoculated and applied nitrogen controls was impracticable. The sites were fertilized to make the soils complete in all nutrients other than nitrogen.

At regular sampling dates, eight separate 12 ft. lengths of row were selected according to a random plan and completely harvested. Because of practical difficulties in handling the large bulk of material at Ormiston, this was reduced later in the season to four rows. At Ayr, one-half of the area was cut at flowering and regrowth studied. Buffer portions were left when sampling. Plant stand at Ormiston was approximately 29 per 12 ft. row, and at Ayr the plants were thinned to five per 12 ft. row.

Data obtained from Ormiston were plant weight, root weight, nodule numbers and weight, and nodule activity. Stem/leaf ratios and nitrogen analyses were obtained on selected samples. Although losses of nodules when sampling were low, soil from around the plant roots was collected when sampling for recovery of nodules and roots which might have been torn off during plant removal. Observations were made also of the distribution of nodules on roots.

Difficulty was experienced in complete removal of roots and nodules at Ayr. Because of this, nodule data from Ayr were considered little better than qualitative and were used only to confirm results from Ormiston.

In both trials soil moisture was adequate for most of the growing season.

Table 1.

FIELD GROWTH AND NODULATION OF *C. pubescens*—ORMISTON, 1956–57.

Date of sampling	6–12–56.	20–12–56.	2–1–57.	15–1–57.	4–2–57.	18–2–57.	11–3–57.	8–4–57.	7–5–57.	26–6–57.
Days after planting	21	35	48	61	81	95	116	144	173	223
Stem and leaf yield	·407*	6·29	13·6	62·3	146·2	368	840	1,047	1,416	730
Stem/leaf ratio	..	..	·16	..	·77	..	·99	..	1·28	3·31
Nitrogen percentage (oven-dry weight)	..	..	3	..	..	..	2·4	..	1·5	1·2
Root weight	·133	1·021	2·640	9·169	15·86	37·25	48·1	71·5	112·7	139·4
Root nitrogen percentage (oven-dry weight)	..	..	1·7	..	..	..	..	..	1·3	1·4
Nodule weight	..	·012	·063	·203	1·066	2·678	4·909	8·350	16·50	11·17
Nodule numbers (including shells)	4·9	18·6	35·2	78	268	315	640	1,030	2,157	1,203
Number of shells	0	0	0	0	0	·5	1·2	37	108	457
Percentage of shells	0	0	0	0	0	·16	·19	3·6	5	38
Mean nodule diameter (mm.)	..	1·4	2·2	2·4	2·8	3·7	3·5	3·5	3·5	3·7
Percentage of active tap-root nodules—										
0–2 mm. diameter	100	100	100	100	89·6	95	70	92·5	(90)	0
3–5 mm. ,,	..	..	..	..	..	91·2	76	64	66·7	5·5
5 mm. ,,	..	..	..	..	..	60	34·8	35·5	15·6	0
Stage of plant growth	..	..	..	..	Running	Nodal rooting	..	Commencement of flowering	..	Frosted
Average minimum screen temperature since previous sampling (°F.)	56·2	59·8	67·2	65·4	65·6	66·2	66·6	60·4	59·8	50·9
Average maximum screen temperature since previous sampling (°F.)	76·8	79·2	88·7	85·0	81·5	82·5	80·4	80·7	79·9	72·5

* All weights are grams oven-dry, per 12 ft. row. Nodule data are on a 12 ft. row basis.

Table 2.

FIELD GROWTH AND NODULATION OF *C. pubescens*—AYR, 1956-57

Date of Sampling	24-12-56.	21-1-57.	18-2-57.	18-3-57.	15-4-57	14-5-57.	11-6-57.	10-7-57.	8-8-57.	18-9-57.	21-10-57.
Days after Planting	41	69	97	125	153	182	210	239	268	309	342
Uncut (1)					flowering						
Stem and leaf yield ..	6·25*	40·8	213	398	618	690	898	904	924	894	971
Stem/leaf ratio	..	0·54	0·76	0·975	1·10	1·35	2·84	2·08	2·45	0·85	0·54
Percentage nitrogen of aboveground parts (oven-dry)	..	..	2·8	..	..	1·9	..	1·3	..	..	2·0
Percentage of active nodules (1–5 mm.) ..	100	100	100	74	55·8	52·3	16·7	Trace	Trace	100	..
(2) *Regrowth after cutting at 154 days*											
Stem and leaf yield ..	..	..	..	..	..	21·5	323	326	346	411	421
Stem/leaf ratio	..	..	..	..	..	1·87	3·49	2·97	2·10	..	·81
Percentage nitrogen of aboveground parts	..	..	..	..	..	1·6	..	..	..	..	2·1
Percentage of active nodules (1–5 mm.)	..	..	..	..	..	47·4	22·9	Trace	..	..	..
Average minimum sreen temperature since previous sampling (°F.)	69·9	72·5	71·4	72·4	65·9	62·4	56·8	54·8	56·5	55·1	59·7
Average mean screen temperature since previous sampling (°F.)	77·9	80·6	79·2	75·3	73·3	70·8	64·5	66·2	64·7	65·8	70·3
Average maximum screen temperature (°F.)	89·9	88·5	88·6	86·1	84·7	84·9	78·3	76·8	76·8	80·8	85·4

* All weights are grams oven-dry, per 12 ft. row. Nodule data are on a 12 ft. row basis.

(2) Results.

The results of the investigations at Ormiston and Ayr are summarised in Tables 1 and 2 respectively. Nitrogen analyses of nodules are given in Table 3.

Table 3.

NITROGEN ANALYSES OF NODULES.

Nodule State.	Nitrogen Percentage. (Oven-dry Weight).
Active (pink)	5·0–5·8
Inactive (green and firm)	5·3
Degenerating (green-brown) ..	4·0
Advanced decay	1·9

(a) Plant Growth.

The oven-dry yields of aboveground parts are shown in Fig. 1 and the growth rates ($\frac{dw}{dt}$) for various intervals in Fig. 2. The growth rate was derived graphically from the growth curve.

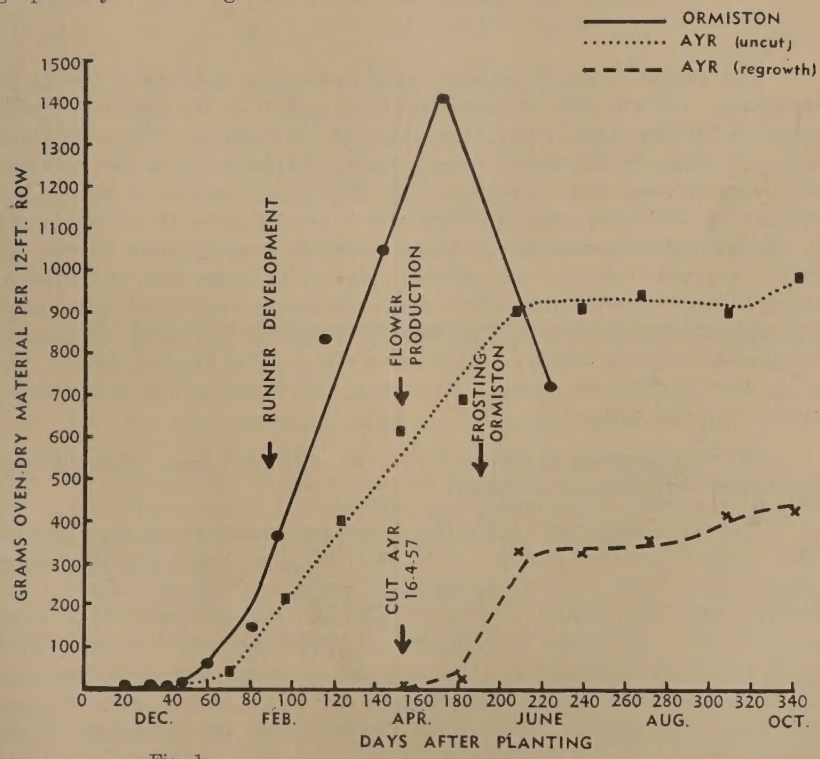

Fig. 1.

Plant Yields (aboveground parts) for *C. pubescens* at Ormiston and Ayr, 1956-57.

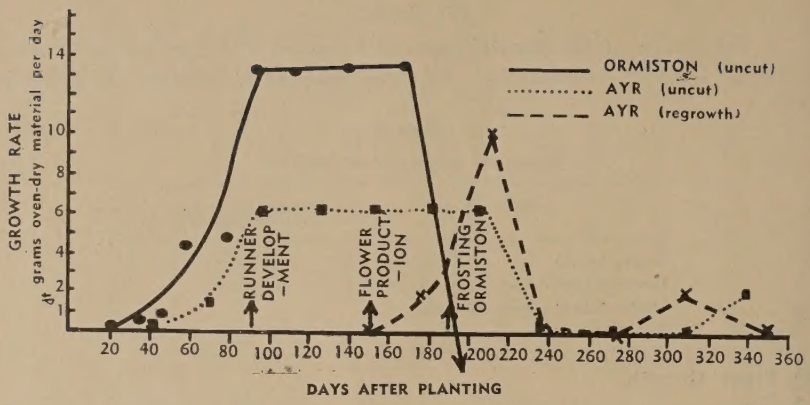

Fig. 2.

Growth Rate Curves for *C. pubescens* at Ormiston and Ayr, 1956-57. The curve for Ormiston reached —15 at 223 days.

The growth curve is sigmoid, yield increasing until early winter to mid-winter. Growth rate increased to a maximum at the time of runner formation (90 days at both sites), after which this maximum level was maintained until early winter to mid-winter, when a decline in growth rate to zero occurred until regrowth commenced in spring. This decline and cessation of growth was associated at both sites with minimum screen temperatures of approximately 55 deg. F. and maximum screen temperatures of approximately 78 deg. F. Growth occurred at Ayr for approximately five weeks longer than at Ormiston. During the winter period, partial or complete defoliation is reflected in increased stem/leaf ratio and lowered nitrogen content of aboveground parts. Defoliation at Ormiston following frosting resulted in a recording of negative growth rate during the 173-223 days period. At Ayr an increased growth rate ensured cutting at flower formation.

Flower production at both sites occurred at 140–150 days. This indicates the absence of a photoperiodic effect.

Net assimilation rates (N.A.R.) have been examined in these studies rather than relative growth rate (R.G.R.) in order to include a photosynthetic factor in the expression of growth. Various workers (e.g., Wilson 1940) have found a relation between photosynthesis, C/N ratio and nodulation. Because of the difficulty of complete recovery of roots at Ayr, N.A.R. at both Ormiston and Ayr was calculated on the aboveground parts alone and not on the whole plant. The general shape of the N.A.R. curve was not altered by this treatment. During the Ormiston trial the percentage of the total weight contributed by the roots varied from 24·8 in the seedling stage (21 days) to 7·4 at the period of maximum yield (173 days).

N.A.R. was calculated by the formula:

$$E_W = \frac{W_2 - W_1}{L_{W_2} - L_{W_1}} \quad 2{\cdot}303 \quad \frac{(\log_{10} L_{W_2} - \log_{10} L_{W_1})}{t_2 - t_1}$$

where E_W = N.A.R. on the basis of leaf weight,

W = Oven-dry weight of the aboveground parts of the plants,

L_W = Oven-dry leaf weight,

t = Time in days.

The N.A.R. for various intervals is shown in Fig. 3.

Despite the maintenance of the high growth rate, N.A.R. declined considerably at both sites from the 80–100 days period (runner formation) until it was quite low at 170–180 days. Regrowth in spring at Ayr is reflected by increasing N.A.R. The N.A.R. curve for both sites is of a similar shape and

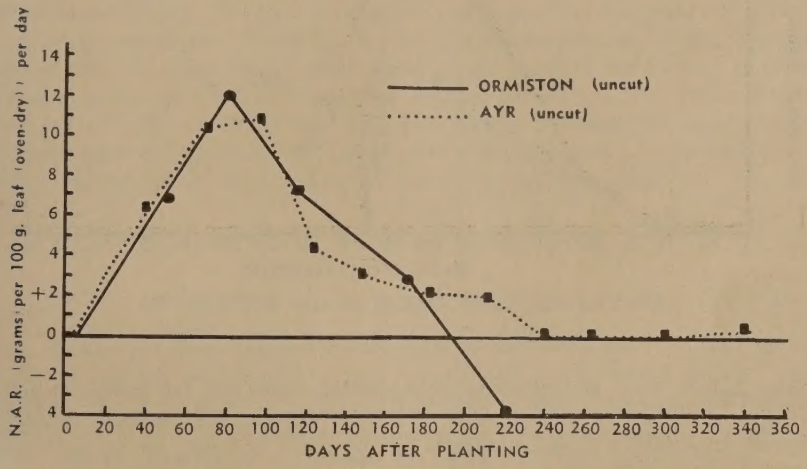

Fig. 3.

Net Assimilation Rates (N.A.R.) for *C. pubescens*, 1956-57.

order. At both sites R.G.R. was also almost identical and declined from 21 days (the earliest observation). Greater yield and growth rate at Ormiston than at Ayr was undoubtedly due to the denser plant stand at Ormiston. This is also reflected in the leaf area indices.

Leaf area indices (L.A.I.) are shown in Fig. 4. These were calculated as the ratio of the area of leaf surface to the ground area.

The shape of the L.A.I. curves closely follows that for yield. Since the leaves of *C. pubescens* are more or less horizontally disposed, one would expect complete light interception at an L.A.I.of 4–8. An L.A.I. of 4 did not occur at Ayr until the second season and at Ormiston until 5–6 months after planting. Closer spacings than the usual 4 ft. might be advantageous in producing higher yields in

the first season by means of more effective interception of light. The highest L.A.I. for a pure stand of *C. pubescens* observed by the author has been 12. This was in an ungrazed stand 15 months old in southern Queensland. The climbing habit of *C. pubescens* may lead to much higher L.A.I. in a mixed pasture.

Root growth increased throughout the period of the trial at Ormiston. The production of stoloniferous roots from the runners is dependent to a great extent on the presence of adequate surface soil moisture. In the Ormiston trial, stoloniferous roots made up 16 per cent. of the total root weight after 173 days. A considerable portion of the total root weight is made up of old taproot,

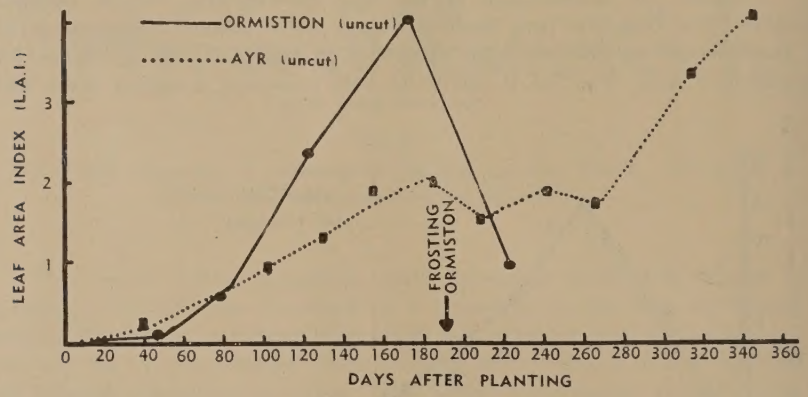

Fig. 4.

Leaf Area Indices (L.A.I.) for *C. pubescens*, 1956-57.

so the shallow, finer, stoloniferous roots probably represent far greater than 16 per cent. of the active feeding roots. This stoloniferous rooting habit enhances efficient exploitation of soil nutrients by the plant and may be an important reason for the ability of some tropical legumes to grow vigorously in soils with a relatively low nutrient status. The advent of stoloniferous rooting makes the plants more independent of the taproot system, except perhaps in times of moisture stress.

In a mixed pasture the contact of runners with the ground is somewhat less and therefore a decrease in stoloniferous rooting could be expected.

Observations at various sites show that smaller roots are lost in mid-winter after plant growth almost ceases. The course of the Ormiston investigation did not allow data on this to be quantitatively assessed. It is probably of the same order as roots lost on cutting (see next section).

(b) Nodulation.

Nodulation continues throughout the active growing period of the plant. During the dormant or slowly growing winter phase, trace infection takes place, the nodules remaining small but active.

Total nodule weight also increases up to the time of cessation of plant growth. Average nodule size (derived from mean nodule weight) increases up to 100 days. This coincides with stolon formation and stoloniferous rooting.

Nodule activity, judged by the presence or absence of haemoglobin on samples of nodules of different sizes, indicates that the larger nodules (greater than 5 mm. diameter) progressively become inactive, while a high percentage of the smaller nodules remain active until plant growth ceases. The data on activity and size of nodules at Ormiston on various dates in no way suggests a progressive elimination of smaller nodules at any stage of plant growth as recorded by Pate (1958a) for *Pisum arvense* L.

After runner formation and stoloniferous rooting the first-formed taproot nodules decay. Nitrogen fixation is increasingly aided by nodules forming on stoloniferous roots, so plant growth from the runners resembles several almost independent plants. With the slowing of growth rate, the percentage of defunct nodules increases also. Cessation of plant growth causes inactivation of almost all nodules. In these studies estimates were not made of the amount of active nitrogen-fixing tissue at each date for possible correlation with plant growth. The author (Bowen 1959) has reported that in nitrogen-free sand culture in glasshouse investigations on *C. pubescens* a highly significant correlation was found between plant yield and weight of nitrogen-fixing tissue.

III. EFFECT OF CUTTING ON NODULATION.

While the investigations reported above gave data on plant and nodule behaviour under unmolested growth conditions, which would obtain when growing plants as a cover crop, no quantitative data were recorded on the effect on nodulation of cutting or damaging the plants. In another study rows of *C. pubescens* were cut to simulate the effects of heavy grazing.

Eight 13 ft. rows of *C. pubescens* planted on Jan. 30, 1957, in the grey-sandy soils of the wallum-heath at Coolum (latitude 26·5°S) were selected. In early May half of each row was cut back to 1 in. above ground level. Eight days and 30 days after cutting, four rows were dug and root weight, nodule numbers and nodule activity recorded.

The results are presented in Table 4.

(1) Plant Growth.

Regrowth occurred after cutting but was small. The most outstanding result of cutting is the marked loss of approximately two-thirds (by weight) of the root system at the 30-day sampling. Plant root loss after cutting was also observed in the growth trial at Ormiston.

Table 4.

EFFECT ON NODULATION OF CUTTING *C. pubescens*.

A. Mean Percentage Active Nodules 8 Days after Cutting.

Nodule Diameter. (mm.).	Plants Cut.	Plants Uncut.
2	23·2	71·6
3	15·8	74·5
4	9·2	54·1
5	4·6	25·8

B. Mean Number of Nodules per Plant after Cutting.

Time (days).	Plants Cut.	Plants Uncut.
8	27·9	34·3
28	7·36	40·5

C. Mean Root Weight per Plant (oven-dry) 28 days after Cutting.

Cut	31 g.
Uncut	90·7 g.

(2) Nodulation.

One week after cutting, a very large percentage of nodules in all size categories became defunct. These were missing at the 30-day sampling. By this time some nodulation was taking place, as was plant regrowth. Most of the nodules were small and were new infections, as evidenced by 60–70 per cent. active nodules in the 1–2 mm. category, compared with 23 per cent. eight days after cutting. Wilson (1942) recorded loss of nodules by a number of legumes on cutting.

IV. DISCUSSION.

A marked periodicity in nodulation of *C. pubescens* is obvious. This has been recorded for other legumes (Allen and Allen 1958; Beeley 1940).

Allen and Allen (1958) noted various causes for nodule loss. One such cause is plant fruition. In recent studies, Pate (1958a, 1958b) noted a great loss of nodules of field pea (*Pisum arvense* L.) and vetch (*Vicia sativa* L.) at flowering. This loss could be offset by prolonging the vegetative phase of growth by disbudding. In the present investigations on *C. pubescens* nodule inactivation commenced 30–60 days before flower production, in the period of declining N.A.R., and reached a maximum at the time of minimum plant growth (also minimum N.A.R.). This was approximately 60–80 days after flowering. Nodule inactivation in this species and possibly other legumes is more fundamentally correlated with plant growth rates or with N.A.R. than with

flowering itself. Inactivation and subsequent loss of nodules seems to result from vegetative growth checks, whether these are due to environmental or physiological causes or to direct damage, rather than to these factors directly.

Pate (1958a, 1958b) found that nodule initiation of field pea and vetch ceases well before flowering. This did not occur with *C. pubescens* in the present studies. The production of nodules paralleled the production of roots. Both nodule and root production continued during and after flowering. The differences from Pate's results may be due to the stoloniferous rooting habit of *C. pubescens*. With the production of stoloniferous roots, nodulation becomes confined very largely to these—little more takes place on the taproot system in that season. Growth from stolons is partially independent of the taproot system. Under the conditions of this investigation, maximum growth per unit area was not attained in the first season. Closer planting would have resulted in increased growth, stoloniferous rooting and nodulation, and hence nitrogen fixation, per unit of land. At the commencement of the second season the stolons are already formed, and maximum growth may occur early in the season. Under these conditions nodulation may reach an early peak and not extend over a considerable period, as in the first season.

The close correlation between course of plant growth and nodule activity indicates that increase of plant growth either directly or by extending the growing season, through the agencies of plant breeding or crop management, should increase nodulation and nitrogen fixation. The course of plant growth determined nodule initiation and longevity and not *vice versa*.

These studies have given an indication that growth of *C. pubescens* declines or ceases when the minimum screen temperature is below 55 deg. F. and the maximum screen temperature less than 78 deg. F. The author has observed that spring regrowth occurs when these temperatures are exceeded. However, in Queensland surface soil moisture is often low in spring, and although plant regrowth occurs the low surface soil moisture impedes nodulation of stoloniferous roots and presumably restricts nitrogen fixation. The present studies suggest that where irrigation facilities are available application of water when seasonal temperatures are below those mentioned may not give a yield increase, but a liberal application when these temperatures are approached in spring will result in unimpeded nodulation of stoloniferous roots and enhanced nitrogen fixation.

The absence of uninoculated plots, and of a comparative series of *C. pubescens* with nitrogenous fertilizers supplied at various levels, prevents conclusions being made on the amount of nitrogen fixed in the growth trials at Ormiston and Ayr. Very few data of this nature have been forthcoming for tropical legumes growing in the field. Akhurst (1954) claimed that, compared with bare ground, the nitrogen increase in the top 7 in. of soil under a cover of *C. pubescens* in one year in Malaya was the equivalent of one ton of sulphate of ammonia per acre (4 cwt. N per acre). Watson (1957) from pot work claimed fixation equivalent to 210 lb. of nitrogen per acre in five months.

Calculations based on nitrogen analyses (Tables 1, 2 and 3) show that after four months' growth the nitrogen content of plant parts at Ormiston were: aboveground parts 40·5 lb. N per acre; roots 1·2 lb. N per acre; and nodules 0·53 lb. N per acre. At six months these were 43·4 lb., 3·1 lb. and 1·6 lb. respectively. At Ayr after four and six months the content of nitrogen per acre in the aboveground parts was 19·1 lb. and 24·3 lb. respectively. In a 15-months-old stand on a nitrogen-deficient wallum-heath soil north of Brisbane, the nitrogen content of tops and roots was 134 lb. and 35·6 lb. per acre respectively. (In this case some nitrogen would have been lost from seasonal loss of plant parts prior to sampling.) Allen and Allen (1958) stated: "Conservative estimates of annual fixation of nitrogen by all legumes average 80 to 100 lb. nitrogen per acre per annum." Remembering that climax growth was not attained in the first season in the present investigations because of wide row spacing and, at Ayr, low plant density, the data above indicate that fixation of nitrogen by *C. pubescens* would be approximately of the order stated by Allen and Allen. According to data presented by Allison (1954), fixation supplying, for example, 100 lb. of nitrogen to the plant may be equivalent to 125–250 lb. of nitrogen applied to soil as an inorganic fertilizer. The difference in general growth and environmental conditions between Malaya and the sites used in these studies may account for the large discrepancy between results reported here and those of Akhurst (1954) and Watson (1957).

Provided no direct excretion of nitrogen occurs from nodules and good growth conditions are maintained, the data here indicate that in an ungrazed state organic nitrogen will be donated to the soil (and thence to associated plants) in small quantities shortly after runner formation. This will come partly from loss of nodules but mainly from loss of fine roots. The amounts will initially be only small, but they will increase to a maximum at approximately one month after cessation of plant growth in winter. (At this stage leaf drop will also be at a maximum.) The rates at which nitrogen will be made available to associated plants will depend on the effect of existing soil conditions on decomposition and nitrification of the organic matter.

Under a cutting or grazing system, organic nitrogen return to the soil by loss of roots and nodules can be induced at will.

However, the roots and nodules together contain only approximately 4–20 per cent. of the total nitrogen content of the plant, so the major return of nitrogen to the soil will be from return of aboveground parts either directly by leaf drop or indirectly by way of the grazing animal. While nitrogen will be added to the soil more quickly under a grazing system, the total amount of nitrogen fixed in a season may vary from the ungrazed, depending on growth as influenced by management and stocking rate. Further studies are needed on nitrogen fixation in relation to grazing management.

The very small amounts of nitrogen contained in the nodules compared with other plant parts are consistent with the now accepted theory of continual

translocation of fixed nitrogen from the nodule to the plant. The finding of very small amounts of nitrogen in the nodules is in direct contrast to the estimate of Butler and Bathurst (1957, pp. 168-178) of 72 lb. N/acre/annum being available through nodule decomposition in perennial clover and ryegrass swards, but corroborates the findings of Pate (1958a). Nodule decomposition releases only very small amounts of nitrogen to the soil. Roots contribute much more.

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SPECIFICITY AND NITROGEN FIXATION IN THE RHIZOBIUM SYMBIOSIS OF *CENTROSEMA PUBESCENS* BENTH.

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SUMMARY.

The plant and bacterial interactions in legume symbiosis have been studied for the tropical legume Centrosema pubescens **Benth. and incidental data on nodulation and symbiosis of other tropical legumes also obtained.**

C. pubescens **and** C. plumieri **(Turp.) Benth. were shown to be highly specific in their bacterial requirements and were more so than** Vigna sinensis **(L.) Endl. ex Hassk.,** Phaseolus lathyroides **L.,** Desmodium uncinatum **(Jacq.) DC.,** Glycine javanica **L. and** Stylosanthes gracilis **H.B.K. The infectivity results are discussed in relation to the classical concept of "cross-inoculation" groups. The cowpea and some other cross-inoculation groups would be better placed in a general tropical legume miscellany within which smaller groups show some relationships between their members.**

Twenty-one Rhizobium isolates from C. pubescens **from different parts of Queensland were tested in sterile sand culture in the glasshouse. A range of effectiveness was recorded and a highly significant correlation was obtained between weight of nodules per plant and aboveground plant weight. Differences between strains in mean nodule weight and numbers of nodules were found. Nodule production by some strains varies markedly with small changes of the environmental conditions. Further studies showed no interference with the nodulation of a highly infective strain, inoculated to the seed, from a non-infective strain added to the soil in heavy concentration.**

The agricultural significance of promiscuity and specificity in nodulation is discussed. The legume with highly specific requirements, while being difficult to establish without inoculation by selected strains, may be more free of competition effects when inoculated seed is sown.

I. INTRODUCTION.

Centrosema pubescens Benth., a legume native to South America, has been used in many tropical and subtropical environments as a cover crop. In Queensland, however, it is becoming increasingly important as a legume component of tropical and subtropical pastures, particularly on well-drained coastal and sub-coastal soils with an annual rainfall greater than 45 in. a year. A study has been made of a number of aspects of the Rhizobium symbiosis of this legume, this paper dealing with infection and plant response to inoculation.

II. INFECTION.

In the classical concept of cross-inoculation groups, *C. pubescens* would be placed in the cowpea group on the ability of isolates from this host to nodulate cowpea and other members of this group (Beeley 1938; Ishizawa 1954) and the occasional nodulation of *C. pubescens* by isolates from members of the cowpea group (Wilson 1939a; Ishizawa 1954).

In some areas of Queensland, *C. pubescens* has failed to nodulate even when planted among well-nodulated native legumes regarded as belonging to the cowpea "cross-inoculation" group. Such observations as this led to studies to define the degree of specificity of this legume for nodulation. Two studies were conducted on *C. pubescens* and other tropical legumes, using isolates from a range of tropical legume genera. A third was conducted at the intrageneric level.

(1) Experiment 1. Field Studies on Cross-inoculation with Isolates from Four Tropical Legumes.

This investigation was carried out at Coolum (latitude 26·5°S), in the wallum-heath coastal area of southern Queensland. The vegetation of the site in the virgin state included native legumes belonging to the cowpea "cross-inoculation" group (Bowen 1956).

Surface-sterilized seeds of *C. pubescens, Desmodium uncinatum* (Jacq.) DC., *Glycine javanica* L. and *Stylosanthes gracilis* H.B.K. were inoculated with an isolate from each of these species. These were planted in 11 ft. rows, using six randomised replications.

Fourteen weeks later inspection of the roots provided the data summarised in Table 1.

Table 1.

Nodulation of Introduced Tropical Legumes in the Wallum Area.

Inoculum Treatment.	Host of Isolation.	Test Plant.			
		Desmodium uncinatum.	*Centrosema pubescens.*	*Glycine javanica.*	*Stylosanthes gracilis.*
No inoculum		−*	−	±	+
QA573 ..	*Desmodium uncinatum*	+	−	±	+
QA616, 1a ..	*Centrosema pubescens* ..	+	+	+	+
QA619, 2a ..	*Glycine javanica* ..	+	−	+	+
CB103 ..	*Stylosanthes gracilis* ..	+	−	+	+

* + = Good nodulation.
− = No nodulation.
± = Poor nodulation of a minority of plants.

The need for inoculation of introduced tropical legumes is indicated, as well as the more specific demands of *C. pubescens*. Inclement growth conditions precluded obtaining conclusive data on relative effectiveness of the various strains. Nodules of *G. javanica* produced by naturally occurring organisms were frequently of an ineffective type. Nodulation occurring on all other species was of an effective type, judged by the presence of haemoglobin. Naturally occuring legume bacteria were able to nodulate *Stylosanthes gracilis*. Other observations have indicated that this legume is not highly specific in its nodulation requirements.

(2) Experiment 2. Glasshouse Tests with 27 Isolates from Tropical Legumes.

The second investigation was conducted in the glasshouse, using a modification of the method devised by Leonard (1943) for sterile sand culture testing of Rhizobium strains. Isolates from 27 tropical legumes were inoculated to *Vigna sinensis* (L.) Endl. ex. Hassk., *Phaseolus lathyroides* L., *Centrosema pubescens* and *C. plumieri* (Turp.) Benth. Twenty-five of these isolates were from legumes not of the genus *Centrosema*. The presence of unusual hypertrophies on the roots of *C. pubescens* necessitated repetition of this portion of the trial. The formation of these hypertrophies was confirmed. The results of Trial 2 are summarised in Table 2.

Table 2.

RESPONSE OF *Vigna sinensis, Phaseolus lathyroides, Centrosema pubescens* AND *C. plumieri* TO INOCULATION WITH ISOLATES FROM TROPICAL LEGUMES.

Strain.	Host of Isolation.	Test Plant.			
		Vigna sinensis.	*Phaseolus lathyroides.*	*Centrosema pubescens.*	*Centrosema plumieri.*
QA374 ..	*Cajanus cajan* (L.) Millsp. ..	+*	+	h*	—
QA549 ..	*Centrosema pubescens* Benth. ..	+	+	+	+
QA616, 1a	,, ,,	+	+	+	+
QA560 ..	*Clitoria ternatea* L.	+	+	h	—
QA705, 1a	*Cyamopsis tetragonoloba* (L.) Taub.	+	+	+h	+
QA638 ..	*Desmodium sessilifolium* (Torr.) Torr. & Gray	+	+	h	—
QA623, 1a	*Desmodium uncinatum* (Jacq.) DC.	+	+	h	—
QA744 ..	,, ,,	+	+	h	—
QA780 ..	,, ,,	+	+	h	+
QA720 ..	*Desmodium* sp.	+	+	h	+
QA618, 2a	*Glycine javanica* L.	+	+	h	—
QA619, 2a	,, ,,	+	+	h	—
QA721 ..	*Glycine max* (L.) Merr.	+	+	h	—
QA626, 3b	*Glycine tabacina* (Labill.) Benth. ..	+	+	h	—
QA636 ..	*Indigofera hirsuta* L.	+	+	h	—
QA641 ..	*Lespedeza hirta* (L.) Hornem. ..	+	+	—	—
QA766 ..	*Lespedeza striata* Hook. & Arn. ..	+	+	h	+
QA629, 1a	*Phaseolus lathyroides* L.	+	+	h	—
QA706c ..	*Phaseolus mungo* L.	+	+	h	—
QA548b ..	*Pueraria phaseoloides* Benth. ..	+	+	h	+
QA759 ..	,, ,,	+	+	h	+
QA760 ..	*Pueraria thunbergiana* (S. & Z.) Benth.	+	+	h	—
QA323 ..	*Pultenaea villosa* Willd.	+	+	h	+
QA751 ..	*Stizolobium deeringianum* Bort. ..	+	+	h	—
QA546b ..	*Stylosanthes gracilis* H.B.K. ..	+	+	h	+
QA752 ..	,, ,,	+	+	h	—
QA753 ..	,, ,,	+	+	+	+
	Uninoculated (quadruplicate) ..	—	—	—	—

* + = Presence of nodules.
h = Presence of root hypertrophies.
— = Absence of nodules and root hypertrophies.

The results show the quite specific requirements of *C. pubescens* and *C. plumieri* and the ability of *V. sinensis* and *P. lathyroides* to nodulate with a wide range of isolates. All observed nodulation was of an effective type except for QA705,1a on *C. pubescens* and *C. plumieri,* which was ineffective. Plants bearing root hypertrophies were identical in growth with uninoculated plants.

The histological nature of the hypertrophies shows them to be modified lateral roots. Despite the absence of organised bacterial tissue in them, their absence from uninoculated pots suggests that they are probably stimulated by the presence of the bacteria. Variation between trials in percentage of plants carrying these hypertrophies and the absence of the phenomenon in the field may point to an aborted infection under the ideal glasshouse testing conditions. They will be discussed more fully by the author in a later paper.

(3) Experiment 3. Glasshouse Study with Isolates from Three Species of Centrosema.

This experiment was at the intrageneric level. The method was the same as that used in Experiment 2. Two isolates from *Centrosema pubescens* (QA549 and QA616,1a), three from *C. plumieri* (QA811a, QA813, and QA821) and one from *C. virginianum* (L.) Benth. (QA860) were inoculated to *C. pubescens* and to *C. plumieri,* using four plants per pot and five replications. All strains from *C. pubescens* and *C. plumieri* nodulated both test species well. However, nodulation by the isolate from *C. virginianum* was very poor and did not occur in all pots. All uninoculated pots were free of nodules. *C. virginianum* is a North American species whereas the other two are South American.

(4) Discussion.

The specific requirements of both *C. pubescens* and *C. plumieri* have been shown. This serves to corroborate the data of Beeley (1938), Wilson (1939) and Ishizawa (1954). E. M. Hutton (personal communication) has found *C. pubescens* to be closely self-pollinated, with possibly some ability for cross-pollination in tropical environments. *C. pubescens* thus fits into the hypothesis proposed by Wilson (1939b) of correlation between cross-pollination and a promiscuous nodulating habit, and between self-fertility and specificity in nodulation. On the other hand, Norris (1956) points out that the promiscuously nodulating *Phaseolus lathyroides* has been found by Hutton to be closely self-fertile.

Although reasonably completely reciprocal nodulation is usual with some of the classical cross-inoculation groups, a close examination shows that these groups appear to be based on a minority of species within genera which are closely related botanically (and often geographically). The results reported here support the evidence of other workers that the strict concept of mutual cross-inoculation cannot be applied to the cowpea "cross-inoculation" group. This is so much so that its members, together with the soybean cross-inoculation

group and possibly the lupin group, the bean group and the strain-specific Sesbania series, would be better regarded as a tropical legume miscellany within which some individual species may nodulate with a wide range of legume bacteria of the cowpea type (promiscuous legumes) and others with very few (highly specific legumes). Within this miscellany, studies within the one genus, e.g. *Phaseolus, Lupinus, Sesbania* and now *Centrosema,* do indicate some degree of order based on botanical and possibly ecological and geographical relationships of small groups. The agricultural significance of promiscuity and specificity is discussed later in this paper.

III. EFFECTIVENESS.

McKnight (1949) gave data showing the low effectiveness of isolates from a number of Queensland native legumes tested on *Vigna sinensis.* Examples of poor effectiveness of many naturally occurring organisms were quoted by Harris (1954).

In some parts of Queensland, *C. pubescens* has become established without inoculation. Such stands are usually well nodulated, the source apparently being from chance infection from naturally occurring organisms. The effectiveness of these naturally occurring strains for *C. pubescens* in Queensland was considered an important aspect for study.

(1) Experiment 1. Relative Effectiveness of 21 Isolations from C. pubescens.

Isolations were made from nodules of *C. pubescens* nodulating naturally in 21 sites from 11 localities in Queensland, from the southern border (latitude 28°S) to Cairns (latitude 17°S). Surface-sterilized seed was planted in sterile testing units of the Leonard type. After emergence, these were thinned to four plants per pot and each plant watered at the base with 1 ml. of a heavy suspension of the test organism. There were six randomised replications. Planting was carried out on Nov. 11, 1957, inoculation 14 days later (7 days after emergence) and harvesting 50 days after inoculation. The first signs of nitrogen fixation were observed 18 days after inoculation. The results are summarised in Tables 3 and 4.

(a) Plant Growth.

Variation in plant growth caused by a plant genetic factor which resulted in poor and often delayed nodulation necessitated two statistical analyses—one based on the 4 plants per pot and the other on the early-nodulating plants as indicated by the presence of well-developed nodules on the crown. Strain QA839 was eliminated from the analyses because of very poor and variable nodulation. Strain QA855 was excluded also because of large individual plot variation.

The results of Experiment 1 show a wide range of relative effectiveness not only among strains collected from widely different areas but also among those collected in the same general area, some strains being up to 60 per cent. more effective than others, under the particular experimental conditions. However, excluding the very poorly infecting strain QA839, all strains are highly effective compared with the uninoculated plots. The order of growth differences between inoculated and uninoculated plants is shown in Fig. 1.

(b) Nitrogen Content.

The analyses show the applied nitrogen controls (equivalent of 1 cwt. ammonium sulphate per acre) to have a much lower nitrogen percentage than inoculated plants. The applied nitrogen was quickly used by the plants, which grew vigorously until the nitrogen was depleted and then grew only slowly. Total nitrogen in all plots, except QA839, exceeded that of the nitrogen added at the equivalent of 1 cwt. of ammonium sulphate per acre.

Table 3.

Relative Effectiveness of Naturally Occurring Rhizobia Strains from *C. pubescens*.

(*a*) *Oven-dry Weight of Plant Tops.*

Strain.	Locality of Isolation.	Mean Weight in Grams per Plant.	Mean Weight Early Nodulating Plants.
Applied Nitrogen ..		0·584	0·584
QA845	Mackay	0·516	0·566
QA851	Utchee Creek (Innisfail)	0·502	0·530
QA616, Ia	Cairns	0·501	0·532
QA549	Utchee Creek (Innisfail)	0·496	0·562
QA850	Utchee Creek (Innisfail)	0·496	0·561
QA836	South Johnstone	0·477	0·477
QA835	Atherton Tableland	0·466	0·485
QA842	Gayndah	0·462	0·555
QA854	East Barron	0·448	0·460
QA837	Ayr	0·439	0·419
QA849	Rockhampton	0·432	0·452
QA841	Gin Gin	0·421	0·421
QA847	Gympie	0·401	0·419
QA843	Mackay	0·400	0·400
QA848	Rockhampton	0·390	0·450
QA844	Mackay	0·390	0·390
QA853	East Barron	0·364	0·403
QA838	Ayr	0·358	0·390
QA852	Rockhampton	0·330	0·339
S.E. Strain Mean		0·040	0·044
Necessary Difference for Significance (5% level)		0·114	0·122
(1% level)		0·150	0·162
QA839	Ayr	0·089	0·109
QA855	Currumbin	0·371	0·399
Uninoculated ..		0·071	0·071

(*b*) *Nitrogen Analyses of Plant Tops.*

Treatment.	Percentage N Based on Oven-dry Weight.
Effectively nodulated	2·9
Applied nitrogen (control)	1·4
Uninoculated	1·2

(c) Number and Weight of Nodules.

These data are presented in Table 4. Three distinct groups are indicated with respect to infection under the conditions of this experiment, namely QA854–QA851, QA842–QA849 and QA839 in Table 4. Strain QA839 was very poorly infective, producing nodules on only half of the test plants. Of these, nodulation was late on half, and numbers of nodules on all nodulated plants was few, the average being 7·5.

There are also significant differences in mean nodule weight.

Table 4.

RELATIVE EFFECTIVENESS OF NATURALLY OCCURRING RHIZOBIA STRAINS FROM *C. pubescens*.

(a) Mean Number and Weight of Nodules.

Strain.	Locality of Isolation.	Nodule Numbers per Plant.		Nodule Weight (g.).
		Transformed Means*.	Equivalent Means.	
QA854 ..	East Barron	1·472	29·7	·00129
QA836 ..	South Johnstone	1·443	27·7	·00163
QA835 ..	Atherton Tableland	1·391	24·6	·00144
QA549 ..	Utchee Creek (Innisfail)	1·377	23·8	·00152
QA847 ..	Gympie	1·362	23·0	·00140
QA855 ..	Currumbin	1·357	22·8	·00121
QA843 ..	Mackay	1·333	21·5	·00168
QA851 ..	Utchee Creek (Innisfail)	1·318	20·8	·00193
QA842 ..	Gayndah	1·221	16·6	·00186
QA844 ..	Mackay	1·218	16·5	·00180
QA850 ..	Utchee Creek (Innisfail)	1·214	16·4	·00221
QA838 ..	Ayr	1·209	16·2	·00180
QA616, 1a ..	Cairns	1·197	15·7	·00262
QA853 ..	East Barron	1·194	15·6	·00173
QA852 ..	Rockhampton	1·181	15·2	·00270
QA841 ..	Gin Gin	1·169	14·8	·00240
QA837 ..	Ayr	1·146	14·0	·00286
QA848 ..	Rockhampton	1·109	12·8	·00213
QA845 ..	Mackay	1·088	12·2	·00269
QA849 ..	Rockhampton	1·045	11·1	·00275
S.E. Strain Mean		·071		·000231
Necessary difference for significance (5% level) ..		·199		·00065
(1% level) ..		·264		·00086
QA839 ..	Ayr		3·7	·00114

* Prior to analyses of the number of nodules per plant the log transformation was used to equalise the variances.

(b) Nitrogen Analyses of Nodules.

4·82% N based on oven-dry weight.

Fig. 1.

Response to Inoculation in Glasshouse Studies. Left: Inoculated. Right: Uninoculated.

(d) Correlation of Plant Growth and Nodule Weight.

Chen and Thornton (1940) showed from considerations of the structure of effective and ineffective nodules of clover, pea and soybean that differences in plant response to these two types of association were due to differences in volume of active bacterial tissue rather than inherent physiological ability of the bacterial strain to fix nitrogen. Four bacterial strains were studied with clover, two with soybean and two with pea. Although this work is often referred to by later workers, similar enquiries into the bases of plant response to different bacterial strains are few.

In the present studies with 17 strains, individual *C. pubescens* plants were harvested and nodule weights recorded for each plant. The average regression of plant weight on nodule weight was found to be highly significant. Differences between regression coefficients for each strain were not significant. The average regression coefficient was 9·09.

This supports the findings of Chen and Thornton (1940) in that the different strains have the same nitrogen-fixing ability weight for weight of active bacterial tissue, the differences in effectiveness resulting from the different amounts of active bacterial tissue formed.

(2) Experiment 2. Isolations from Three Species of Centrosema.

Isolations from *C. pubescens, C. plumieri* and *C. virginianum* were tested on *C. pubescens* and *C. plumieri,* using the modified Leonard technique and five replications. The experiment was planted on Jan. 13, 1958, inoculated 7 days later, and harvested 34 days later still.

The results are presented in Table 5.

All strains except QA860 were effective on *C. plumieri.* While the applied nitrogen treatment (equivalent of 1 cwt. ammonium sulphate per acre) produced the highest plant weight, the nitrogen percentage of plants in this treatment was so low that it was exceeded by the total nitrogen from plants of all strains except QA860. The poor effectivity of QA860 was found to be due to low infection and not lowered nodule size. All strains were effective on *C. pubescens,* QA549 being superior to the others. However, QA860 was poorly infective here also.

The trial did not proceed long enough for total nitrogen in inoculated plots to exceed that of the applied nitrogen treatment. Some tendency in that direction was observed.

(3) Experiment 3. Combined Inoculation of Effective and Non-infective Strains.

Experiments 1 and 2 on effectiveness were carried out using a pure culture method. In field practice this may not be the case. The author (Bowen 1956) has indicated that almost all of the studied indigenous legumes of

Table 5.

EFFECTIVENESS OF RHIZOBIA STRAINS ON *C. plumieri* AND *C. pubescens.*

(*a*) *Oven-dry Weight of Plant Tops.*

1. *C. plumieri.*

Strain.	Host of Isolation.	Mean Oven-dry Weight of 4 Plants (g.).
Nitrogen		3·14
QA821	*C. plumieri*	2·97
QA616, 1a ..	*C. pubescens*	2·92
QA549	*C. pubescens*	2·76
QA813	*C. plumieri*	2·68
QA811, a	*C. plumieri*	2·54
QA860	*C. virginianum*	1·20
Uninoculated ..		0·94
S.E.		0·251
Necessary difference for significance (5% level) ..		0·73
(1% level) ..		0·98

2. *C. pubescens.*

Strain.	Host of Isolation.	Mean Oven-dry Weight of 4 Plants (g.).
Nitrogen		1·82
QA549	*C. pubescens*	0·73
QA811, a	*C. plumieri*	0·58
QA813	*C. plumieri*	0·54
QA821	*C. plumieri*	0·51
QA616, 1a ..	*C. pubescens*	0·48
QA860	*C. virginianum*	0·40
Uninoculated ..		0·28
S.E. (excluding the nitrogen control)		0·065
Necessary difference for significance (5% level) ..		0·19
(1% level) ..		0·26

(*b*) *Nitrogen Analyses of Plant Tops.*

Treatment.	Percentage Nitrogen Based on Oven-dry Weight.	
	C. plumieri.	*C. pubescens.*
Effectively nodulated	3·0	3·0
Applied nitrogen (control)	1·6	2·1
Uninoculated	1·3	1·4

Queensland belong to the tropical legume miscellany with respect to infection. In many areas of Queensland, large numbers of native legumes occur. There is therefore in many places a soil flora carrying high populations of legume bacteria of the tropical legume type. This raises the question of possible interference to nodulation by native Rhizobium strains incapable of forming nodules on the host.

Krasilnikov and Karenyako (1944) and Harris (1954) have reported a stimulative effect on nodulation by other soil organisms. Purchase and Nutman (1957) reported that with avirulent and virulent clover strains the avirulent strain competed with the virulent strain and in mixtures containing a great excess of the avirulent form the formation of nodules was reduced. Kronberger (1936) also found a depression of plant response using a mixed culture.

Experiment 3 was carried out using a sterile sand culture and the modified Leonard technique. QA323, an isolate from the native legume *Pultenaea villosa* Willd. and non-infective on *C. pubescens*, was inoculated to the sand at $3x10^4$ organisms per gram of sand just prior to planting surface-sterilized seed of *C. pubescens* inoculated with the effective strain QA549 at the rate of $1{\cdot}4x10^3$ organisms per seed. Twelve seeds per pot were planted and the seedlings were thinned to 4 plants per pot. Sixteen replications of QA549 alone, QA323 alone, and the mixture were employed.

At harvest 11 weeks later no significant differences were recorded in either plant weight or nodule numbers between QA549 alone and the QA549–QA323 mixture. Mean oven-dried plant weights per pot were 0·674 g. and 0·668 g. respectively and nodule numbers per pot 20·4 and 18·2 respectively. QA323 alone produced no nodulation and was equivalent to uninoculated controls, for which the mean oven-dried plant weight per pot was 0·137 g.

(4) Discussion.

In general, Rhizobium strains isolated from naturally infected plants of *C. pubescens* and *C. plumieri* proved to be effective on *C. pubescens*. Although the small typical ineffective type of nodule has been produced in the glasshouse by the author with a few selected isolates from other tropical legumes, such typical ineffective nodules have not been seen in the field.

In Experiment 1 a highly significant correlation was shown between individual plant growth response to nodulation and the total weight of nodules on the plant. Comparing the 17 bacterial strains examined, differences in the regression coefficients for dependence of plant weight on nodule weight were not significant. This shows that differences in effectiveness between the bacterial strains were due only to differences between strains in the amount of active bacterial tissue formed and not to differences between strains in the nitrogen-fixing ability per unit weight of bacterial tissue. The statistical analysis of data in Table 4 indicates that some of the more effective strains produced significantly more nodules under the experimental conditions while other highly effective strains achieved a high total nodule weight by producing larger nodules.

The very poor effectivity of strain QA839 is explained by a very poor production of nodules (i.e., low infection) and a slight decrease in mean nodule weight. Again, in Experiment 2, poor response of *C. pubescens* and *C. plumieri* to QA860 is mainly a matter of poor infection. Ishizawa (1955) recorded ineffective responses in *C. pubescens,* but a number of strains giving such responses were shown earlier (Ishizawa 1954) to be poorly infective. Examination of the data of other workers (McKnight 1949) on *Vigna sinensis* shows that poor plant response is often a result of poor infective ability of the Rhizobium.

It may be asked, "How absolute are differences in relative effectiveness?" The virulence (or ability to produce nodules) of a strain varies under varying conditions. Other workers have shown that a number of soil physical, chemical and biological factors and climatic factors can modify number of nodules. The results of Experiment 2 indicate strain QA549 to be significantly better on *C. pubescens* than QA616,1a at the 5 per cent. significance level. The difference approaches 1 per cent. significance, yet response to the two strains was practically identical in Experiment 1. The difference in Experiment 2 was caused by very poor production of nodules by QA616,1a under the particular set of experimental conditions. Mean nodule weights of QA616,1a and QA549 were of the same order. Other such cases have been recorded in this work.

A need is indicated for a closer study of the property of infection and nodule formation and of plant physiological and environmental factors affecting these. Meanwhile, the selection of a legume bacterium for a particular legume for general agricultural use must depend on the result of trials under several conditions, with an eye to selection of one of high effectiveness and consistently high virulence as suggested by Raju (1936). Such a strain is QA549 for *C. pubescens.*

The mutually effective nodulation of *C. plumieri* and *C. pubescens* by strains effective on either is interesting, considering the very specific requirements of *C. pubescens* for infectiveness and effectiveness. These are both South American species and these results lend further support to the possibility of establishing minor effectiveness sub-groupings within the "tropical legume miscellany."

The agricultural significance and importance of promiscuity and specificity may be an interesting field for further study. The ability of *Vigna sinensis* to nodulate effectively with a wide range of legume bacteria has been one factor in its great agronomic popularity. (Conversely, this popularity has led to its wide use as a test plant for Rhizobium isolates from many tropical legumes and their designation to the cowpea "cross-inoculation" group.) Similarly, the promiscuous *Phascolus lathyroides* has been a popular pasture plant in Queensland for some years. On the other hand, some legumes nodulate promiscuously but the association is usually poorly effective. This can lead to quite misleading conclusions regarding the plant's potential. Such legumes appear to be *Samanea saman* (Jacq.) Merrill and *Clitoria ternatea* L. (Allen and Allen 1939).

When considering the mixed culture interaction, the position with establishing *C. pubescens* in the field in Queensland is that it is highly specific in its requirements for nodulation but when nodulating with naturally occurring strains of bacteria such association is likely to be effective, the actual response depending on the environment. Experiment 3 indicates that interference with a highly infective inoculated strain by naturally occurring legume bacteria of the cowpea type not infecting *C. pubescens* is not likely to be of importance. This is in accordance with the findings of Erdman (1946) and Hofer (1945). It is possible that response to strains of variable or low virulence may be reduced by such naturally occurring soil organisms.

Leonard (1930), Dunham and Baldwin (1931), Nicol and Thornton (1941), Burton and Allen (1949) and Harris (1954) have shown that mixtures of poorly effective and highly effective strains may result in an intermediate plant reaction or even an ineffective response. Harris (1954) states—"Where the soil already supports a considerable population of rhizobia, irrespective of whether effective or ineffective types predominate, it is only with difficulty that the desired strain can be superimposed on the natural pattern by seed inoculation."

It would seem that the legacy of the promiscuously nodulating legume is one of possible interference to highly effective inoculated strains by poorly effective naturally occurring legume bacteria. This would be of more economic importance in a perennial legume with a stoloniferous habit. Many pasture legumes fall into this category. The legume with more specific bacterial requirements, while needing inoculation for establishment and display of its potential to plant introduction officers and farmers alike, may be more free of insidious competition effects. More intensive study at the mixed culture level is urgently needed.

IV. PLANT HETEROGENEITY.

The presence of very poorly nodulated plants in the field side by side with heavily nodulated plants has been observed by the author since the commencement of these studies in 1955. This has been observed with seed from several countries and with all strains of legume bacteria for *C. pubescens* studied in the field. In the Rhizobium strain trials in pots, Leonard jars and test tubes, a number of plants failed to nodulate, while others nodulated only sparsely and often only on distal roots despite the presence in the same pot of well-nodulated, vigorously growing plants.

This phenomenon was further investigated, starting with vegetatively propagated material of both well and poorly nodulated plants. Evidence accumulated suggests that a plant factor exists which determines the extent of nodulation in *C. pubescens*. This work will be described in more detail in a subsequent paper.

V. ACKNOWLEDGEMENTS.

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FRENCH BEAN VARIETY AND SPACING TRIAL.

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SUMMARY.

As part of a regional testing programme, two new rust-resistant varieties of French bean—Redlands Belle and Redlands Beauty—were compared with recognised commercial varieties in a plant and row spacing trial at the Maroochy Experiment Station.

Yields from both new varieties were considerably greater than those of Brown Beauty and comparable with those of St. Andrews.

Plant weight at maturity and number of flowers per plant increased with increments in plant spacing within the row from 2 in. up to a maximum of 6 in.

Differences in percentage pod set were obtained for varieties but not for plant spacings in and between rows. Maximum pod set occurred in St. Andrews.

Weight loss in cased beans during a 6-days storage period was influenced by variety rather than the tightness of the pack.

I. INTRODUCTION.

For some years past, the green bean industry has been based primarily on the old-established variety, Brown Beauty. Types of lesser importance are St. Andrews at Gympie and Hawkesbury Wonder at Stanthorpe.

The varietal pattern remained fairly stable until 1954, when bean rust (Strain 17A) was first recorded in Queensland. The existing commercial varieties proved highly susceptible to the disease and plant breeders therefore took immediate steps to produce substitute varieties with agronomic properties comparable with those of Brown Beauty, and with the added character of resistance to rust. Their appreciation of the threat implicit in the introduction of rust proved sound, and by 1957 the disease had become a major limiting factor to bean production in certain districts during the winter and spring months.

One outcome of the plant-breeding project was the production of two new varieties, known respectively as Redlands Belle and Redlands Beauty, both of which were bred at the Department's Redlands Experiment Station. These were listed for regional testing in 1957 and one of several trials was assigned to the Maroochy Experiment Station, near Nambour, which is an important green bean growing district on the Near North Coast, some 75 miles north of Brisbane. In order to get the maximum amount of information from the trial, the scope of the work was extended to permit an investigation of the effects of row spacing and plant spacing in the row. Both vary considerably in commercial plantings and are influenced by soil type, the implements used for cultivation and the availability or otherwise of water for irrigation.

II. EXPERIMENTAL METHODS.

(1) Treatments and Design.

Row spacings were 2 ft. and 2½ ft.; and plant spacing in the row 2 in., 4 in. and 6 in.

The varieties used were Brown Beauty, Redlands Beauty, Redlands Belle, St. Andrews and Langshaw Beauty.

The layout was a (2 x 3 x 5) x 3 factorial with split plots for row spacings. Sub-plots comprised 4 x 11 ft. rows in the 2½ ft. spacing and 5 x 11 ft. rows in the 2 ft. spacing. Adjacent plots were separated by buffer areas at the ends and by guard rows at the sides.

(2) Cultural Data.

The land selected for the trial was a red-brown sandy loam which had been previously under pigeon pea for several years. Pre-planting tillage brought the soil into a good tilth before the seed was sown on Apr. 24, 1957.

Crop management followed standard practice. Planting furrows were opened up at the prescribed spacings before planting and a 4:15:2 complete fertilizer distributed by hand along the bottom and sides of the furrow at the rate of 8 cwt. per acre. The fertilizer was covered with 2 in. of soil by hand-hoe immediately. The seed was sown by hand at the specified spacings to a depth of 1 in. and the land irrigated shortly afterwards. A side-dressing of sulphate of ammonia at the rate of 1 cwt. per acre was applied on May 10, some two weeks after germination.

Weeds developing in the row were smothered by drawing soil into the plant row from the inter-row space. Weed growth in the inter-row spaces was controlled by hand-tillage implements.

The crop was grown in relatively dry weather with supplementary irrigation as and when necessary (Table 1).

Table 1.

Climatic Data.

Month.	Rainfall (in.).	Irrigation (in.).	Mean Maximum Temperature (°F.).	Mean Minimum Temperature (°F.).
From Apr. 24	·02	3	81·5	60·8
May	·09	7	75·5	51·1
June	1·56	4	70·0	51·3
To July 17	1·74	Nil	66·5	42·0

Pests and diseases were not troublesome. Bean fly (*Melanagromyza phaseoli*) was effectively controlled with DDT (0·1 per cent.) sprays applied at intervals of approximately three days for a period of four weeks after germination. Bean rust was present, though not in sufficient quantity to reduce yield in the more susceptible varieties.

The green pods were harvested in five picks from June 27 to July 17, and sold on the fresh vegetable market.

III. VARIETAL CHARACTERISTICS.

All five of the varieties under test have distinctive features. Brief descriptions are given below:—

Brown Beauty.—Bush relatively small with rather dense foliage; crop borne mostly near the centre of the plant and close to the main stem; pods of fine quality when picked at the right stage of maturity, flat and dark-green in colour. Cropping period comparatively short.

Redlands Belle.—Derived from Langshaw Beauty (a string bean locally selected in 1954 from Brown Beauty at Gympie) and Florida Belle (a stringless bean introduced to Queensland some years ago). Bush semi-erect and compact but somewhat larger than that of Brown Beauty; pods borne mainly in the centre of the plant, semi-flat in cross section and tending to develop fibre in transit to distant markets. Matures ahead of Brown Beauty; short cropping period. Resistant to rust but somewhat susceptible to stem rot.

Redlands Beauty.—Derived from Brown Beauty x Californian Small White (a navy bean). Bush erect and open with spreading laterals and somewhat distinct, narrowly hastate leaves; pods uniformly flat in cross section, dark-green in colour and rather disperse on the plants. Moderately long cropping period. Resistant to rust and anthracnose.

St. Andrews.—The strains currently grown in Queensland are mainly local selections which differ somewhat from the type. Plant with an erect bush and strong laterals; pods long, flattish, pale-green in colour and tending to cling to the plant when mature. Long cropping period. Reputed to be tolerant of low temperatures and low soil fertility.

Langshaw Beauty.—A selection from Brown Beauty but quite distinct from that variety. Bush large, semi-open, with dark-green leaves; pods very long, carried well out from the centre of the plant and dark-green in colour. Long cropping period.

IV. RESULTS.

(1) Yields.

Yield data from the trial are summarised in Table 2.

Yields from Redlands Belle, Redlands Beauty and St. Andrews were 32 per cent. greater than those from Brown Beauty, while Langshaw Beauty produced 17 per cent. more beans than Brown Beauty. Differences were significant at the 1 per cent. level and were independent of plant spacing or row spacing.

Table 2.

MEAN YIELDS (oz./plot).

Variety.	Plant Spacing.				Row Spacing.		
	2 in.	4 in.	6 in.	Mean.	2 ft.	2½ ft.	Mean.
1. Brown Beauty	329	325	284	313	322	304	313
2. Redlands Belle	415	410	412	412	423	401	412
3. Redlands Beauty ..	430	409	400	413	408	419	413
4. St. Andrews	412	432	389	411	422	399	410
5. Langshaw Beauty ..	351	357	391	366	372	361	366
Mean	387	387	375	..	389	377	..

Significant differences: 2, 3, 4>> 5>> 1

Yield differences are an expression of varietal characteristics such as plant size, cropping habit, pod weight and climatic adaptability. The pattern of yields for the varieties under test followed expectations, but the recorded differences may be accentuated by the relatively low yield from the standard variety, Brown Beauty. This variety may be under-valued by the data, for the seed was slow to germinate (Table 3) and this may indicate lack of vigour in the stock due to factors such as age of the seed and/or storage conditions prior to planting.

Table 3.

FIELD GERMINATION OF VARIETIES.

Time.	Brown Beauty.	Redlands Belle.	Redlands Beauty.	St. Andrews.	Langshaw Beauty.
Within 7 days ..	35	80	75	71	78
„ 14 „ ..	77	89	86	84	83

The lack of significant differences in yield for plant spacings in the row is surprising. Overseas plant-spacing experiments with lima beans and dwarf French beans indicate that increased yields per acre were obtained with closer planting and increased seeding rates. The results of this trial suggest that the ability of the plants to exploit the environment is much the same at 2, 4 and 6 in. spacings. The environment sets a limit to plant development, and plant development, in turn, is frequently directly correlated with productivity.

Assuming an effective lateral root penetration of 8 in. into the inter-row space, the mean area occupied by each plant ranged from 32 sq. in. for the 2 in. spacings to 92 sq. in. for the 6 in. spacing where the rows are 2 ft. apart.

The absence of significant differences in yield for row spacings ranging from 2 ft. to 2½ ft. may reflect the restricted lateral root spread in a quick-growing crop produced under irrigation.

(2) Plant Weight.

Within the limits set by the constitution of the variety, plant weight at any given spacing is a measure of land utilization. It was determined in this trial from 4-plant per plot samples taken at random immediately before plants had shown any obvious signs of senescence. The results are summarised in Table 4.

Table 4.

GREEN WEIGHT AFTER HARVESTING (oz./4 plants).

Variety.	Plant Spacing.				Row Spacing.		
	2 in.	4 in.	6 in.	Mean.	2 ft.	2½ ft.	Mean.
1. Brown Beauty	7·8	11·5	11·6	10·3	9·7	10·9	10·3
2. Redlands Belle	7·1	13·9	16·8	12·6	11·9	13·4	12·6
3. Redlands Beauty ..	8·8	14·8	15·9	13·2	13·5	12·8	13·2
4. St. Andrews	8·3	13·9	16·6	12·9	12·0	13·9	12·9
5. Langshaw Beauty ..	7·6	11·0	15·8	11·5	11·0	11·9	11·5
Mean	7·9	13·1	15·3	..	11·6	12·6	..

Significant differences:
Varieties: 3,4 >> 1; 2 > 1.
Plant spacing: 6 in. >> 4 in. >> 2 in.

The plant-weight data draw attention to varietal differences in plant size. Redlands Beauty, St. Andrews and Redlands Belle plants were 9 per cent. larger than Langshaw Beauty and approximately 25 per cent. larger than Brown Beauty. Both Redlands Beauty and St. Andrews normally develop strong laterals. Redlands Belle has a more erect bush which is morphologically similar to that of Brown Beauty. Its good showing in Table 4, which is indicative of greater plant size, may reflect greater tolerance of cold temperatures during the growing period than is characteristic of Brown Beauty.

The effect of plant density in the row on vegetative development was very marked. Doubling the space between plants from 2 in. to 4 in. increased plant weight by some 65 per cent., and trebling it from 2 in. to 6 in. by 93 per cent. The initial increase in plant weight of the first increment (2–4 in.) in spacing is much greater than for the second (4–6 in.).

(3) Number of Flowers.

Each flower represents a potential pod and the flowering characteristics of a variety are therefore of considerable importance in beans as in other horticultural crops. The fate of the flowers will, of course, depend on environmental factors such as climate, nutrient status of the soil and soil moisture, but differences in the capacity of the plant to produce flowers could materially affect yields under any given set of conditions. Flower counts were therefore made on four plants taken at random from each plot. The data are summarised in Table 5, which expresses the results in terms of equivalent numbers.

Table 5.

Number of Flowers (per 4 Plant Sample).

Variety.	Plant Spacing.				Row Spacing.		
	2 in.	4 in.	6 in.	Mean.	2 ft.	2½ ft.	Mean.
1. Brown Beauty	98	135	166	130	139	122	130
2. Redlands Belle	79	146	180	127	133	122	127
3. Redlands Beauty ..	99	168	173	142	136	148	142
4. St. Andrews	97	158	194	144	138	150	144
5. Langshaw Beauty ..	98	144	207	146	147	144	146
Mean	94	150	185	..	138	137	..

Significant differences: 6 in. >> 4 in. >> 2 in.

The number of flowers per plant was much the same in all varieties but significant differences were recorded for the three plant spacings used in the row. Increasing the spacing from 2 in. to 4 in. was followed by a 59 per cent. increase in the number of flowers per plant, and from 2 in. to 6 in. by a 95 per cent. increase. The product of "number of flowers per plant" and "number of plants per unit distance" was much the same at 2 in. and 4 in. spacings.

Soil moisture and nutrient levels were for practical purposes uniform throughout the experimental area. Vegetative growth and flower production could, however, vary at different levels of soil moisture and nutrient supply as there is some evidence with other crops that plant density and utilization of applied nutrients are correlated.

Row spacing had no significant effect on the number of flowers produced. With normal methods of inter-row cultivation, root competition between plants in adjacent rows is unlikely to take place when, as in this trial, water is supplied in adequate amounts and fertilizer is placed in a readily accessible position.

(4) Pod Setting.

As the bean plant is normally self-pollinated, variations in pod set are due primarily to the effect of climate and nutrition on pollination. Responses to these factors may be inherent in the variety. Pod set (Table 6) was determined from random samples of four plants per plot.

Plant spacing in the row and spacing between rows had no effect on the percentage pod set.

Significant differences were, however, recorded between the varieties grown, pod set being lowest in Brown Beauty (58·4 per cent.) and highest in St. Andrews (79·6 per cent.). The other varieties occupied intermediate positions. The differences may reflect varietal reactions to low temperatures, for poor setting is a common aftermath of chilling winds during the winter months.

Table 6.

POD SET (PERCENTAGE/4 PLANTS/PLOT).

Variety.	Plant Spacing.				Row Spacing.		
	2 in.	4 in.	6 in.	Mean.	2 ft.	2½ ft.	Mean.
1. Brown Beauty	62·0	64·4	48·6	58·4	56·0	60·7	58·4
2. Redlands Belle	71·0	67·6	70·5	69·7	67·6	71·8	69·7
3. Redlands Beauty ..	63·4	67·6	61·5	64·2	63·8	64·5	64·2
4. St. Andrews	86·1	76·9	75·8	79·6	81·8	77·4	79·6
5. Langshaw Beauty ..	63·7	66·8	62·0	64·1	63·3	65·0	64·1
Mean	69·2	68·6	63·6	..	66·5	67·9	..

Significant differences: Variety 4 >> 2, 3, 5 >> 1.

(5) Storage Losses.

A considerable proportion of the Queensland bean crop is consigned to distant markets in hessian sacks or wooden cases. Weight loss between packing on the farm and display at markets may therefore be high. Such losses affect returns to growers partly because weight has a direct influence on market values, and partly because shrinkage tends to expose the beans to greater injury in transit. Weight losses were therefore determined in loose, medium and tightly packed cases during a 6-days storage period.

Each case contained a 4 lb. sample of each variety packed in random order. Cases were also packed top and bottom with extra beans in order to adjust weight to 59 lb. (loose), 64 lb. (medium), 68 lb. (tight), and held at room temperatures. The results are summarised in Table 7.

Table 7.

PERCENTAGE WEIGHT LOSS IN CASES AFTER 6 DAYS.

Variety.	Pack.			
	Lower.	Medium.	High.	Mean.
1. Brown Beauty	8·5	9·6	8·0	8·7
2. Redlands Belle	10·0	10·2	7·3	9·2
3. Redlands Beauty	7·1	6·8	7·9	7·3
4. St. Andrews	8·5	7·2	7·6	7·8
5. Langshaw Beauty	8·9	10·9	6·5	8·8
Mean	8·6	9·0	7·5	8·3

Significant differences: 2, 5 > 3.

Weight loss bore no significant relation to the type of pack, although trends in the data strongly suggest that the tighter the pack the less the loss in weight during storage. This would be expected owing to the reduced air movement in and through the cases.

There are, however, significant differences in weight losses between Redlands Belle and Langshaw Beauty on the one hand and Redlands Beauty on the other. It is of interest in this connection to recall that Langshaw Beauty is one of the parents used in the production of Redland Belle and that Redlands Belle, unlike other varieties used in this trial, has pods with an oval rather than a flat cross-section. Percentage weight loss was lowest in Redlands Beauty.

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EFFECT OF MATURITY AND TREE AGE ON THE BEHAVIOUR OF QUEENSLAND GROWN DELICIOUS APPLES STORED AT 34-36°F.

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SUMMARY.

Investigations were conducted over a period of four years to determine the effect of maturity and tree age on the cool storage behaviour of Queensland grown apples of the Delicious variety.

The main disorder encountered was superficial scald, which was extremely severe in fruit picked in February. Later picking dates resulted in almost complete absence of the disorder. As a general rule, fruit from young trees was more susceptible to storage disorders than that from old trees.

Of the picking dates studied, those in the first week in March resulted in long storage with fewer disorders than the earlier picking dates.

In order to avoid severe losses due to withering in the store, the fruit should be removed from storage before the end of September.

I. INTRODUCTION.

In view of a large increase in planting in the post-war period, it has become most important to the Queensland apple industry that the marketing life of apples be spread over as long a period as possible to absorb the increased production.

A series of experiments was initiated in 1953 to study the effect of time of picking and tree age on the storage behaviour of the Delicious variety grown on the Granite Belt and held under refrigerated conditions. The investigations were continued each year until 1956. Progress has been briefly reported in the Annual Reports of the Queensland Department of Agriculture and Stock for the years 1953-54 to 1956-57. More extended reports have appeared in *Queensland Fruit and Vegetable News*, a weekly newspaper published for fruit growers by the Queensland Committee of Direction of Fruit Marketing. In addition, the 1955 investigations were fully reported by Stevenson (1957) as part of a report on apple cool storage investigations for that year.

II. EXPERIMENTAL DETAILS.

The experimental fruit for each year was obtained from the same six orchards on the Granite Belt surrounding Stanthorpe. These orchards were selected as representative of the different growing environments within the

area. Blocks of old and young trees were selected in each orchard and all fruit used in these investigations was harvested from these trees. The ages of the young trees varied between 6 years and 13 years and those of the old trees between 19 years and 30 years. In 1953, two picking dates were studied but in the three subsequent years three picking dates were used. The dates were as shown in Table 1.

Table 1.

PICKING DATES.

Pick.	1953.	1954.	1955.	1956.
First	..	Feb. 17	Feb. 14	Feb. 16
Second	Feb. 24	Feb. 25	Feb. 23	Feb. 23
Third	Mar. 5	Mar. 9	Mar. 7	Mar. 6

Three half-bushel cases from each picking date were taken from each of the blocks of old and young trees in each of the six orchards—i.e. 12 half-bushel cases from each orchard in the 1953 experiment and 18 half-bushel cases from each orchard in the subsequent experiments.

After picking, the fruit was wrapped in oiled paper wraps and stored at 34–36 deg. F. Removals of fruit from store were made on three occasions each year. After removal from store, the cases were held at air temperatures for seven days to simulate normal marketing conditions, and then inspected for storage disorders.

Firmness was measured by means of a Magness penetrometer. Five fruits from each case were taken as a sample and readings were made on opposite sides of the fruit with the $\frac{7}{16}$ in. plunger. The mean of the 10 readings was recorded.

III. RESULTS.

The results are summarised in Table 3. It will be noted that removal dates of the experimental fruit varied from year to year, but that they fell into five definite groupings, which for ease of comparison have been designated Removals 1 to 5. Actual removal dates are shown in Table 2.

Table 2.

DATES OF REMOVAL OF EXPERIMENTAL FRUIT FROM STORE.

Removal.	1953.	1954.	1955.	1956.
1	..	..	Aug. 1	July 30
2	Aug. 31	Aug. 30	Sept. 5	Aug. 27
3	Sept. 14	..	..	..
4	..	Oct. 4	Oct. 3	Oct. 2
5	Oct. 19	Oct. 20	..	..

IV. DISCUSSION.

(1) 1953 Results.

No superficial scald was present in the fruit examined and only a small number of fruit was affected by mould.

The total wastage consisted mainly of fruit affected by radial water core. This disorder was described by Carne (1948). It develops in ripening fruit, and even though cool storage will delay its onset, the fruit often develops breakdown while in store. If it does not develop as breakdown in store, it usually causes rapid deterioration of the fruit when it is removed at atmospheric temperatures.

More breakdown was encountered in fruit from young trees than in fruit from old trees, large fruit from young trees being particularly susceptible. Fruit from the first pick (Maturity 2) was less affected by breakdown than that from the second pick (Maturity 3). There was no evidence of immaturity disorders and the fruit remained in a fairly satisfactory condition until Oct. 19, 1953 (Removal 5) and subsequently for a week under atmospheric conditions.

The firmness of the fruit on removal was affected by maturity, storage time and age of the tree from which it was harvested. Fruit remained firmer the earlier it was picked and decreased in firmness with increase in the length of the storage period. Fruit from old trees was firmer than that from young trees.

(2) 1954 Results.

In view of the absence of immaturity disorders in fruit from the 1953 investigations, for the 1954 investigations in addition to the two picking dates previously used—viz., Maturity 2 and Maturity 3—an earlier pick was made on Feb. 17, 1954 (Maturity 1).

Some superficial scald was present in fruit from these investigations, particularly in fruit from the first pick. This effect of maturity on the incidence of superficial scald has been reported for the Granny Smith variety by Tindale and Huelin (1939) and Stevenson (1957).

Total wastage consisted mainly of fruit affected by mould and superficial scald. Internal radial water core, which constituted a large part of the total wastage in the 1953 investigations, affected very few fruit. A trend which indicated a reduction in the total wastage present with increase in maturity of the fruit was observed. However, this trend was not significant when the results were analysed. Fruit from young trees had less wastage than that from old trees and total wastage increased with increase in the length of the storage period.

The firmness of the fruit on removal was affected by maturity, storage time and age of tree. Fruit remained firmer the earlier it was picked and decreased in firmness with increase in the length of the storage period. Fruit from old trees was firmer than that from young trees.

Table 3.

ADJUSTED MEAN PERCENTAGES OF DEFECTS AND FIRMNESS OF DELICIOUS APPLES AFTER REMOVAL FROM COOL STORE.

	Mould.				Superficial Scald.				Soggy Breakdown.				Internal Radial Water Core.				Withered Fruit.*				Total Wastage.†				Firmness.			
	1953	1954	1955	1956	1953	1954	1955	1956	1953	1954	1955	1956	1953	1954	1955	1956	1953	1954	1955	1956	1953	1954	1955	1956	1953	1954	1955	1956
Removal 1—																												
Maturity 1	..	..	0·5	3·2	..	..	16·3	7·3	..	..	Nil	Nil	..	..	Nil	0·2	..	..	..	2·8	..	..	16·8	10·7	..	..	13·04	11·25
Maturity 2	..	..	1·6	2·2	..	..	4·3	Nil	..	..	Nil	Nil	..	..	Nil	0·5	..	..	..	4·5	..	..	6·2	2·7	..	..	12·58	10·54
Maturity 3	..	..	0·6	4·1	..	..	Nil	Nil	..	..	Nil	Nil	..	..	Nil	0·5	..	..	..	2·6	..	..	0·6	4·6	..	..	11·46	10·04
Old Trees	..	..	0·3	3·6	..	..	3·7	1·5	..	..	Nil	Nil	..	..	Nil	0·6	..	..	..	3·7	..	..	4·0	5·7	..	..	12·08	10·58
Young Trees	..	..	1·7	2·9	..	..	6·7	3·2	..	..	Nil	Nil	..	..	Nil	0·2	..	..	..	2·8	..	..	8·6	6·3	..	..	12·64	10·64
Removal 2—																												
Maturity 1	..	..	0·1	1·2	1·5	..	1·0	34·4	6·8	..	Nil	Nil	Nil	..	Nil	Nil	Nil	..	..	..	4·1	..	1·1	36·0	8·3	..	16·3	14·12	11·33
Maturity 2	..	3·8	0·6	1·0	1·0	Nil	Nil	5·9	2·6	1·8	Nil	Nil	Nil	Nil	0·1	Nil	Nil	..	..	..	5·7	5·6	0·7	6·9	3·6	14·2	15·7	12·62	10·92
Maturity 3	..	0·3	0·4	1·3	3·6	Nil	0·1	0·1	Nil	0·7	0·1	Nil	Nil	Nil	Nil	Nil	Nil	..	..	..	4·9	1·0	0·6	1·6	3·6	13·2	15·3	11·71	10·29
Old Trees	..	0·4	0·2	1·0	2·8	Nil	0·1	7·2	3·0	0·4	0·1	Nil	Nil	Nil	Nil	Nil	Nil	..	..	..	5·8	0·8	0·4	8·4	5·8	14·0	16·0	12·72	10·94
Young Trees	..	3·7	0·6	1·2	1·1	Nil	0·7	13·6	3·4	2·2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	..	..	..	4·0	5·9	1·3	14·8	4·5	13·4	15·6	12·92	10·75
Removal 3—																												
Maturity 1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Maturity 2	..	1·0	..	..	..	Nil	..	..	..	1·3	..	..	..	6·2	..	..	..	..	..	..	..	8·5	..	..	..	13·5	..	..	..
Maturity 3	..	1·7	..	..	..	Nil	..	..	..	0·5	..	..	..	8·6	..	..	..	..	..	..	..	10·9	..	..	..	12·6	..	..	..
Old Trees	..	1·6	..	..	..	Nil	..	..	..	0·2	..	..	..	1·7	..	..	..	..	..	..	..	3·5	..	..	..	13·2	..	..	..
Young Trees	..	1·0	..	..	..	Nil	..	..	..	1·5	..	..	..	13·3	..	..	..	..	..	..	..	15·8	..	..	..	12·9	..	..	..
Removal 4—																												
Maturity 1	..	..	0·7	1·9	1·0	..	2·4	34·5	3·8	..	Nil	Nil	Nil	..	Nil	Nil	Nil	..	..	..	12·0	..	3·1	36·5	4·8	..	15·4	15·21	11·71
Maturity 2	..	..	2·0	2·0	1·9	..	0·6	9·4	0·8	..	Nil	Nil	Nil	..	0·1	Nil	Nil	..	..	..	13·2	..	2·8	11·9	2·7	..	15·0	14·29	11·17
Maturity 3	..	..	1·0	2·0	4·8	..	0·3	0·1	Nil	..	Nil	Nil	Nil	..	Nil	Nil	Nil	..	..	..	8·7	..	1·3	2·6	4·8	..	14·2	12·96	10·83
Old Trees	..	..	0·6	1·4	3·3	..	0·3	8·8	1·3	..	Nil	Nil	Nil	..	0·1	Nil	Nil	..	..	..	14·9	..	1·0	10·6	4·6	..	15·3	13·94	11·28
Young Trees	..	..	1·8	2·6	1·3	..	2·3	15·6	2·3	..	Nil	Nil	Nil	..	Nil	Nil	Nil	..	..	..	7·7	..	4·1	18·5	3·6	..	14·5	14·36	11·19
Removal 5—																												
Maturity 1	..	..	0·4	..	..	..	3·5	..	..	..	0·3	..	..	..	Nil	..	..	..	..	..	..	..	4·3	..	..	..	15·8	..	..
Maturity 2	..	2·4	1·9	..	..	Nil	1·2	..	..	1·8	0·1	..	..	2·3	Nil	..	..	..	..	..	..	6·5	3·2	..	..	12·6	15·6	..	..
Maturity 3	..	0·7	2·8	..	..	Nil	0·1	..	..	5·7	0·6	..	..	6·7	Nil	..	..	..	..	..	..	13·1	3·4	..	..	12·2	12·2	..	..
Old Trees	..	0·9	1·3	..	..	Nil	0·9	..	..	1·5	0·3	..	..	2·3	Nil	..	..	..	..	..	..	4·7	2·5	..	..	12·7	15·4	..	..
Young Trees	..	2·2	2·0	..	..	Nil	2·5	..	..	6·1	0·4	..	..	6·6	Nil	..	..	..	..	..	..	14·9	4·9	..	..	12·1	14·0	..	..

* The percentages of withered fruit in the years 1953, 1954 and 1955 were very small and are not shown.

† Totals for 1956 do not include withered fruit.

(3) 1955 Results.

As in previous investigations, only a small number of fruit was affected by mould and in this year's experiments no internal radial water core was present.

Total wastage comprised mainly superficial scald, which was very severe in fruit from the first pick. Less scald was present in fruit from the second pick, while that from the third pick was not affected. Wastage decreased with increase of maturity at picking and increased with storage time. Fruit from old trees had less wastage than that from young trees.

The firmness of the fruit on removal was affected by maturity, storage time and age of tree. Fruit remained firmer the earlier it was picked. However, two results obtained during this year's investigations did not support previous results—fruit from young trees was firmer than that from old trees, and the fruit increased in firmness with length of storage. These facts cannot be satisfactorily explained, although some shrivelling of fruit did occur, particularly

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Analysis of Table 3.

Significance of Differences in Total Wastage.

1953.

Removal 3 and Removal 5 significantly greater than Removal 2 (5% level).
Young trees significantly greater than Old trees (1% level).

1954.

Removal 5 significantly greater than Removal 2 (5% level).
Young trees significantly greater than Old trees (5% level).

1955.

Removal 2 significantly greater than Removal 1 (5% level).
Removal 4 significantly greater than Removal 1 (1% level).
Young trees significantly greater than Old trees (1% level).
Maturity 1 significantly greater than Maturity 2 and Maturity 3 (1% level).
Maturity 2 significantly greater than Maturity 3 (1% level).

1956.

Removal 3 significantly greater than Removal 1 and Removal 2 (1% level).
Old trees significantly greater than Young trees (5% level).

Significance of Differences in Firmness.

1953.

Removal 2 significantly greater than Removal 5 (1% level).
Removal 3 significantly greater than Removal 5 (5% level).
Removal 2 significantly greater than Removal 3 (5% level).
Old trees significantly greater than Young trees (5% level).
Maturity 2 significantly greater than Maturity 3 (1% level).

1954.

Removal 2 significantly greater than Removal 4 and Removal 5 (1% level).
Old trees significantly greater than Young trees (5% level).
Maturity 1 significantly greater than Maturity 2 and Maturity 3 (5% level).
Maturity 2 significantly greater than Maturity 3 (1% level).

1955.

Removal 4 significantly greater than Removal 2 and Removal 1 (1% level).
Removal 2 significantly greater than Removal 1 (1% level).
Young trees significantly greater than Old trees (1% level).
Maturity 1 significantly greater than Maturity 2 and Maturity 3 (1% level).
Maturity 2 significantly greater than Maturity 3 (1% level).

1956.

Removal 4 significantly greater than Removal 1 (1% level).
Maturity 1 significantly greater than Maturity 2 and Maturity 3 (1% level).
Maturity 2 significantly greater than Maturity 3 (5% level).

with fruit from young trees. It is possible that moisture losses accompanying shrivelling make the fruit more resistant to penetration by the test plunger and this may have accounted for the increasing firmness of the fruit. Shrivelling of apples during storage was discussed by Smock and Neubert (1950, pp. 240-241), who attributed it to too low a relative humidity in the storage chamber. As the fruit used in these experiments was stored in a commercial cool store, with loading and unloading constantly being carried out, control of relative humidity was not possible.

(4) 1956 Results.

Total wastage increased with increase in the length of the storage period, but there were no significant differences in the amount of wastage from young and old trees. Wastage decreased with increase in maturity at picking and increased with length of the storage period. A large number of fruit was badly affected by withering, which became more pronounced with increase in storage time. Maturity had no effect on withering but fruit from old trees was more affected than that from young trees. Superficial scald was moderately severe in fruit from the first pick but disappeared in later picks. The length of storage period had no effect on the incidence of superficial scald. Fruit from old trees was less affected than that from young trees. Only a small amount of mould was present.

V. CONCLUSIONS.

The results obtained from the 1953 experiments indicated that the optimum picking date for long storage of the Delicious variety, grown on the Granite Belt, was between the end of February and the first week in March. In the experiments conducted during the years 1954, 1955, and 1956, when an earlier picking date was used, considerable superficial scald was encountered. This disorder was greatly reduced in the later picks, and from the results obtained in those years, the first week in March appears to be the more suitable, since some scald was present in fruit picked at the end of February.

Throughout the four years' investigation, fruit from old trees generally stored better than that from young trees and was less affected by disorders. When the fruit was held later than the end of September, withering of the fruit became serious and its effect became more apparent the longer the storage period. For this reason it is inadvisable to hold this variety in cool store after the end of September.

VI. ACKNOWLEDGEMENTS.

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post-storage conditions of the fruit. Statistical analyses were made by Messrs. P. B. McGovern and L. N. Balaam (Departmental Biometricians), who also guided the author in the design of the experiment.

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BLUE MOULD *(Peronospora tabacina* Adam*)* OF TOBACCO IN NORTH QUEENSLAND: SOME ASPECTS OF CHEMICAL CONTROL.

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SUMMARY.

The results of screening trials to assess the efficiency of various fungicides and fungicidal mixtures for blue mould control are detailed and discussed. Field spray trials are described and a theory of acquired resistance following blue mould attack is developed to explain the anomalous results in some trials. Evidence drawn from field experiments is presented to substantiate this theory.

I. INTRODUCTION.

Flue-cured tobacco is an important crop in North Queensland. The producing areas in 1958 were the Mareeba-Dimbulah district (4,400 acres), the Burdekin (1,225 acres), and Ingham (150 acres). The average yield is between 700 and 800 lb. of leaf per acre. This compares very unfavourably with the yield of flue-cured tobacco obtained in the United States of America. While it is probable that many factors contribute to this reduced yield, it is plain that the incidence of tobacco blue mould in the field in North Queensland makes substantial inroads on the crop. Estimates of percentage loss on individual farms range as high as 44 per cent. in a crop planted from healthy seedbeds (Angell 1957). The destruction of bottom leaf by spotting is very considerable, but in some seasons, particularly when wind storms are experienced, this loss is aggravated considerably by the collapse of crops in which the incidence of systemic infection is high.

Since the Second World War and consequent on the completion of various water conservation schemes in the tobacco-growing areas, there has been a rapid expansion in the acreage of tobacco grown under irrigation in North Queensland. It was with the increase in the number of irrigated farms that blue mould emerged as an ever-present and serious problem in the field. This appears to be related to the early or spring planting which is now the rule for such crops. The seedbeds are planted in June and July and transplanting takes place during August and September. The plants are subsequently exposed to weather conditions, in the form of low minimum temperatures and frequent heavy dews during September and October, which enable the disease to become established. If rain eventuates the inoculum potential increases rapidly and an epiphytotic develops.

No resistance to the disease has been noted in any of the flue-cured varieties grown on a commercial scale in North Queensland up to the present.

II. EFFECTS OF THE FIELD DISEASE ON THE PLANT.

In the initial stages of infection in the field the plant develops large chlorotic lesions on the bottom leaves. On the lower surfaces of these lesions a purplish down, comprising the conidiophores and conidia, is produced. As the lesions age they become necrotic. Such necrotic spots drop out either in the field or during curing. On badly affected leaves the spots are so numerous as to coalesce; the leaves become entirely chlorotic, develop a practically continuous layer of down on the lower surface, wilt and die very quickly. As the infection progresses the fungus may invade the conducting tissues of midrib and stalk and establish a systemic infection (Angell and Hill 1932).

Systemically infected plants commonly exhibit wilting symptoms and stunted leaves may be produced following vascular penetration. Sucker growth is often abnormal and sucker leaves may be narrow and mottled. The most serious aspect of this phase of the disease is, however, the loss of plants by lodging due to weakening of the infected stems at their bases where vascular invasion is usually most intense.

Subsequent foliage infections on systemically infected plants show as much smaller spots or flecks with greatly inhibited sporulation or as a breakdown of veins often accompanied by puckering of the lamina around the blighted structure.

III. SCREENING OF FUNGICIDES FOR BLUE MOULD CONTROL.

The benzol fumigation treatment of tobacco seedlings (Angell, Allen and Hill 1936) when used as recommended by the Queensland Department of Agriculture and Stock (Pont 1956) has given satisfactory control of the disease in seedbeds in North Queensland. However, with the increase in importance of blue mould as a field problem, it became necessary to investigate the possibility of control by fungicides in the field. As a preliminary stage in this project, the efficiency of a large number of fungicides was assessed in a series of screening trials conducted in the years 1951, 1952, 1956, 1957 and 1958*.

The fungicidal control of blue mould has received considerable attention in the United States. The use of the dithiocarbamates in seedbeds has been well investigated since fermate was first used for that purpose (Anderson 1942), and both ferbam and zineb (sprays and dusts) have been shown to control the disease in that country (Todd 1955).

A cuprous oxide-cottonseed oil treatment was developed for mould control in the United States (Clayton *et al.* 1938). It was later shown that even though the oil was not toxic to spores of the blue mould fungus it made tobacco more resistant to the disease. Cuprous oxide alone was almost ineffective (Clayton *et al.* 1943). These workers also demonstrated that the salicylates as a group were good blue mould fungicides.

* The results of the first 1959 screening trial became available as this article was going to press. The more interesting of these have been included in Tables 1 and 2.

The fungicidal value of the salicylates was further investigated at a later date (Graham *et al.* 1947) and benzyl salicylate again found to give satisfactory results. In addition, the synergism of the cuprous oxide-cottonseed oil mixture used by Clayton *et al.* (1943) was discussed and the synergism of mixtures of salicylates and various other organic fungicides was demonstrated.

Recently mixtures of cottonseed oil with wettable powder suspensions of organic fungicides have been tested for the control of field blue mould in the United States (Rich and Taylor 1957).

Malachite green has been used for the control of downy mildew of onion and suggested as a control for other downy mildews (McWhorter and Pryor 1937). It has also been shown to inhibit sporulation of the onion downy mildew fungus (Yarwood 1941).

(1) Methods and Procedure.

The screening trials were conducted with seedbed plants. Standard sized seedbeds 10 ft. by 4 ft. were used. Each was divided into five plots to give a plot size of 2 ft. by 4 ft. Treatments were replicated three, four or more times, depending on their number. A randomised block layout was used (Fig. 1).

Fig. 1.

A Typical Seedbed Screening Trial.

Treatments were applied twice in seven days—generally on the Monday and Friday of each week while the trial lasted.

Spray treatments were applied with a bucket pump equipped with a single Rega Cyclone nozzle. Spray drift between plots was prevented by enclosing each plot in plywood screens while it was being sprayed. Sprays were applied

from each side of the bed with the nozzle held as nearly parallel to the ground as possible in order to ensure maximum coverage of the lower surfaces of the leaves.

Dust treatments were applied with a Rega Bonza duster. Drift was similarly prevented by enclosure in screens. To ensure maximum coverage the plot being treated was totally enclosed except for the opening on the side from which the dust was being applied. Plots were dusted successively from each side of the seedbed.

When the seedlings had reached a suitable size, generally about five weeks after planting, all beds were inoculated with a strong aqueous suspension of conidia of *Peronospora tabacina*. The beds were watered uniformly with the suspension from a 3 gal. watering can with a fine rose.

An unsprayed check treatment was included in each trial.

Spraying terminated when blue mould was well developed in the check plots, and the results were assessed. This was achieved by counting the number of diseased seedlings in a sample of approximately 150 plucked from the centre of each plot. This figure was then expressed as percentage infection. A rating for intensity of infection was given at the same time. In all cases this rating was found to be closely correlated with the percentage infection figure.

The average percentage infection figure for the check plots was never below 96 and in most trials was between 99 and 100. The difference between treatments was always so obvious that statistical analysis of the data was never necessary.

The results are presented in Tables 1 and 2. In these tables the percentage blue mould infection figures originally obtained have been shown as ratings. The conversion from percentage infection was made as follows: 0=nil; 1=1–10 per cent. infected plants; 2=11–20 per cent. infected plants, and so on to 10=91–100 per cent. infected plants.

The scale used for phytotoxicity rating was:

0	Nil.
+	Scattered necrotic spotting but no setback in growth.
++	More general necrotic spotting. Slight stunting.
+++	Necrosis and stunting without death.
++++	Severe necrosis and stunting. Death of many seedlings.

(2) Discussion.

The results of the screening trials described in this article confirm that copper fungicides used alone are relatively inefficient for blue mould control. In Table 1 it will be seen that none of the coppers had a rating lower than 6. The home-made cuprous oxide mixture used in these trials was the molasses mix

Table 1.

COMBINED RESULTS OF 1951–1959 SCREENING TRIALS USING SINGLE INGREDIENTS.

Fungicide.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Cuprox (50% copper as copper oxychloride)	3·75 lb.	(2) 1951	7	0
		1956	10	0
,,	5 lb.	(1) 1952	9	0
Home-made cuprous oxide (25% copper as cuprous oxide)	3·75 gal.	(3) 1952	6	0
		1956	9	+
		1958	8	0
Copper oxydul ultra (80% copper as cuprous oxide)	4 lb.	(1) 1956	9	++
Duphar Colloidal Copper (15% copper)	6 pints	(1) 1956	9	++
Crag Potato Fungicide (copper zinc chromate)	2·5 lb.	(1) 1956	10	+
Copper Nirit (5% copper —45% thiocyanodinitrobenzene)	3 lb.	(1) 1957	2	+++
Blitox Ipsilon (12% copper)	5 lb.	(1) 1957	7	0
Phygon (50% dichloronaphthoquinone)	1 lb.	(1) 1951	5	0
S.R. 406 (50% captan)	2 lb.	(2) 1951	7	0
		1952	10	0
Fermate (76% ferbam)	4 lb.	(2) 1951	3	0
		1952	7	0
Zerlate (80% ziram)	4 lb.	(2) 1951	4	0
		1952	6	0
Methasan (100% ziram)	4 lb.	(1) 1951	3	0
Fulasin ultra (90% ziram)	1·5 lb.	(1) 1956	2	0
Zebtox (65% zineb)	3·75 lb.	(5) 1956	1, 3	0, 0
		1958	2, 10*	0, 0
		1959	7*	
Curit (72% zineb)	3·75 lb.	(1) 1958	10*	0
Dithane M22 (70% maneb)	2 lb.	(1) 1956	5	0
Dispersible Tetroc (chloranil)	4 lb.	(3) 1957	1	0
		1958	1, 10*	0, 0
TZU. 4311 (40% thiram ; 20% ziram ; 20% tetra methyl dithiocarbamine-acid-methyl arsine)	4 lb.	(1) 1956	1	++++
,,	2 lb.	(1) 1956	6	++++
Shell Fungicide WL 1491 (polymeric thiocarbamate)	3·75 lb.	(1) 1958	10*	0
Phaltan (N trichloromethyl thiophthalimide)	3·75 lb.	(1) 1958	10*	0
Goodrite Zac (zinc dimethyl dithiocarbamate cyclohexidamine)	3·75 lb.	(1) 1956	4	++
P.M.F. (4% w/w mercury as phenyl mercury dinaphthyl methane disulphonate)	0·5 lb.	(1) 1956	5	++
Quat (1% w/v cetyl trimethyl ammonium bromide ; 5% w/v β naphthoxy-acetic acid)	2·5 gal.	(1) 1956	9	Hormone injury
Agrimycin (15% streptomycin ; 1·5% oxytetracycline)	2·34 lb.	(1) 1956	7	+
Hexamine	3 lb.	(1) 1957	9	0
Benzyl salicylate	1 lb.	(1) 1959	6*	0

Combined Results of 1951–1959 Screening Trials using Single Ingredients—*continued*.

Fungicide.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Methyl salicylate	2 lb.	(1) 1959	10*	0
Bismuth salicylate	1·5 lb.	(1) 1959	10*	
Salicylanilide	3·75	(1) 1959	9*	
Bulbosan (7·5% trichlorotrinitrobenzene)	Dust	(3) 1957	1, 2	0, 0
		1959	7*	
Zineb 10%	Dust	(1) 1959	2*	0

* The second 1958 trial and the 1959 trial were severe ones in that conditions for infection and blue mould ev elopm ent were particularly good.

which was at one time the standard recommendation for the control of blue mould in seedbeds in Queensland (Mandelson 1933). Zineb had low ratings in the 1956 trials and in the first 1958 trial. The rating of 10 in the second 1958 trial can be explained by the fact that this was deliberately made a severe test, in which the disease was allowed to become established in the plots before the first spray applications were made. Ziram gave good results in a 1956 trial and ferbam had a low rating in the 1951 test. Another promising organic fungicide was chloranil, which was tried in 1957 and 1958. The thiocyanodinitrobenzene-copper oxychloride mixture used in 1957 gave good control of the disease but was extremely phytotoxic.

The only dust used, viz., trichlorotrinitrobenzene, proved to be extremely efficient in the 1957 trials. The performance of this fungicide was undoubtedly enhanced by the excellent coverage obtained with the method of application described above.

The most interesting features of this series of trials appear in Table 2, where the data for the various fungicide and fungicide-oil mixtures are presented. The chief points are listed below:

(*a*) The increase in fungicidal efficiency gained by using such mixtures is obvious and may be gauged by examining the case of cuprous oxide and its various mixtures. From a consideration of Tables 1 and 2 it is possible to make the following deductions:

(i) Cuprous oxide is less efficient than cuprous oxide plus white oil, which in turn is less efficient than cuprous oxide plus white oil plus malachite green.

(ii) Cuprous oxide plus white oil is less efficient than cuprous oxide plus cottonseed oil, and cuprous oxide plus white oil plus malachite green is less efficient than cuprous oxide plus cottonseed oil plus malachite green.

(iii) Cuprous oxide plus cottonseed oil plus malachite green is less efficient than cuprous oxide plus cottonseed oil plus benzyl salicylate.

Table 2.

COMBINED RESULTS OF 1956–1959 SCREENING TRIALS USING MIXED INGREDIENTS.

Fungicidal Mixture.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Home-made cuprous oxide	3·75 gal.	(1) 1956	4	++
Zebtox	3·75 lb.			
Home-made cuprous oxide	3·75 gal.	(1) 1957	7	++
White oil	2½ pt.			
Home-made cuprous oxide	3·75 gal.	(2) 1957	1	+
Cottonseed oil	6 pt.	1958	4	++
Home-made cuprous oxide	3·75 gal.	(3) 1956	3	+++
White oil	2½ pt.	1957	2,2	+++, +++
Malachite green	1·6 oz.			
Home-made cuprous oxide	3·75 gal.	(3) 1957	1, 1	++, ++
Cottonseed oil	6 pt.	1958	2	+++
Malachite green	1·6 oz.			
Home-made cuprous oxide	3·75 gal.	(1) 1958	1	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Cupravit Blue	5 lb.	(2) 1956	6	++
White oil	2½ pt.	1957	3	+++
Malachite green	1·6 oz.			
Copper oxydul ultra	3 lb.	(1) 1957	0	++++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Copper oxydul ultra	2 lb.	(1) 1957	2	+
Cottonseed oil	6 pt.			
Zebtox	3·75 lb.	(1) 1957	1	0
White oil	2½ pt.			
Zebtox	3·75 lb.	(2) 1957	1	+
Cottonseed oil	6 pt.	1958	2	+
Zebtox	3·75 lb.	(2) 1957	1, 1	++, ++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Zebtox	3·75 lb.	(2) 1957	1	+
Cottonseed oil	6 pt.	1958	2	++
Malachite green	1·6 oz.			
Zebtox	3·75 lb.	(1) 1959	1*	+
Benzyl salicylate	1 lb.			
Zebtox	3·75 lb.	(1) 1959	3*	–
Methyl salicylate	2 lb.			
Zebtox	3·75 lb.	(1) 1959	4*	–
Bismuth salicylate	1½ lb.			
Zebtox	3·75 lb.	(2) 1957	0	0
Cottonseed oil	6 pt.	1958	1	++
Benzyl salicylate	8 oz.			
Zebtox	3·75 lb.	(1) 1958	2	++
Cottonseed oil	6 pt.			
Benzyl salicylate	5·3 oz.			

COMBINED RESULTS OF 1956–1959 SCREENING TRIALS USING MIXED INGREDIENTS—*continued.*

Fungicidal Mixture.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Zebtox	2 lb.	(1) 1958	4	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Zebtox	3·75 lb.	(1) 1959	2*	++
Cottonseed oil	6 pt.			
Benzyl salicylate	1 lb.			
Zebtox	3·75 lb.	(1) 1958	2	+++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Malachite green	1·6 oz.			
Curit	3·75 lb.	(1) 1958	4*	+++
Cottonseed oil	6 pt.			
Benzyl salicylate	12·8 oz.			
Curit	3·75 lb.	(1) 1958	5*	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Curit	3·75 lb.	(1) 1958	3*	++
Cottonseed oil	6 pt.			
Methyl salicylate	3·75 lb.			
Curit	3·75 lb.	(1) 1958	7*	+
Cottonseed oil	6 pt.			
Methyl salicylate	1·8 lb.			
Fulasin ultra	2 lb.	(1) 1957	1	+++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Fulasin ultra	2 lb.	(1) 1957	1	++
Cottonseed oil	6 pt.			
Maneb	2 lb.	(1) 1957	1	+++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Dispersible Tetroc	4 lb.	(2) 1957	1	++
Cottonseed oil	6 pt.	1958	3	++
Dispersible Tetroc	4 lb.	(1) 1958	3	+++
Cottonseed oil	6 pt.			
Malachite green	1·6 oz.			
Dispersible Tetroc	4 lb.	(1) 1958	3	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Benzyl salicylate	8 oz.	(1) 1957	1	0
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.	(1) 1957	1	++
Cottonseed oil	6 pt.			
Malachite green	1·6 oz.			
Zebtox	10%	(1) 1959	2*	++
Zinc salicylate	2% dust			

* This second 1958 trial and the 1959 trial were severe ones in that conditions for infection and blue mould development were particularly good.

(*b*) In the case of the zineb combinations there are indications of similar though less obvious differences in the efficiency of zineb alone and with the various additives

(*c*) As would be expected, the concentrations of the ingredients in the mixture have an effect on its fungicidal efficiency. Zineb (3·75 lb.—100) plus cottonseed oil plus benzyl salicylate (8 oz.—100) is superior to either zineb (2 lb.—100) plus cottonseed oil plus benzyl salicylate (8 oz.—100) or zineb (3·75 lb.—100) plus cottonseed oil plus benzyl salicylate (5½ oz.—100).

(*d*) In most cases the mixtures are more phytotoxic than the constituent fungicides used alone. The phytotoxicity is aggravated particularly by the inclusion of malachite green in the mixture.

(3) Conclusions.

The superiority of the organic fungicides zineb, ziram, ferbam and chloranil over the various copper compounds used in these trials was shown. Improved efficiency resulted from the addition of cottonseed oil, white oil, cottonseed oil plus malachite green, white oil plus malachite green, cottonseed oil plus benzyl salicylate or cottonseed oil plus methyl salicylate to either copper or organic fungicides. Phytotoxicity to tobacco seedlings generally increased with the use of fungicidal mixtures.

This increase in efficiency shown by mixtures was undoubtedly due to the synergism reported by other workers with tobacco blue mould fungicides.

IV. FIELD SPRAY TRIALS FOR BLUE MOULD CONTROL.

During the years 1952 to 1958 a series of field trials of blue mould fungicides was conducted at various sites in the Mareeba-Dimbulah area. The fungicides used in these trials were generally selected after consideration of the results of the screening trials which preceded them. However, when zineb became available in 1954, it was used in a field trial without prior testing, due principally to the reports of its efficacy for blue mould control which were received from the United States.

These field experiments will be considered in two groups; firstly, the experiments of 1952, 1953, and 1954, in the course of which the puzzling behaviour of blue mould fungicides under field conditions first became apparent; and, secondly, the experiments of 1956, 1957, and 1958, in which the problem of control was investigated in more detail.

(1) Methods and Procedure.

The experimental layout was in all cases a randomised block. The plot size varied in the early experiments but in 1956 was standardised at three rows. The centre row was regarded as the datum row for the assessment of results. The length of the row was never less than half-a-chain.

The plants were sprayed when small with a Rega knapsack (Moles Patent type) spray equipped with either a Cyclone nozzle or a Rega No. 6 adjustable nozzle. In the later stages of growth a portable power spray was used. After early trial with various types of nozzles the Rega No. 6 adjustable was chosen as the standard. A pressure close to 100 lb. per sq. in. was used, as pressures greater than this injured tender foliage. The aim of the operator was to get the fungicide on to the lower surfaces of all leaves on the plant.

The main features of the early trials are summarised below, after which the later trials are discussed in more detail.

(2) Field Trial, 1952.

(*a*) *Treatments.*—The 1952 trial consisted of 12 replications of 3 treatments:—home-made cuprous oxide (1½ gal. stock–40 gal.); ferbam(4 lb.–100 gal.); and untreated.

Five applications were made at 7-day intervals. Spraying commenced when the plants were two weeks in the field and terminated four weeks later, which was about four weeks before harvest.

(*b*) *Method of Rating.*—Each plant was rated for blue mould damage at the completion of spraying on a scale ranging from 0 (=complete absence) to 10 (=complete defoliation). The results appear in Table 3.

Table 3.

Mean Rating for Blue Mould Damage in 1952 Field Trial.

Treatment.	Mean.	Sig. less than (1% level).
A. Cuprous oxide	1·15	C
B. Ferbam	0·72	A, C
C. Nil	2·01	

(3) Field Trial, 1953.

(*a*) *Treatments.*—Eight replications were made of 6 treatments:—home-made cuprous oxide (1½ gal. stock–40 gal.), primed and unprimed; 80 per cent. ziram (2 lb.–100 gal.), primed and unprimed; and unsprayed, primed and unprimed.

Four applications were made at 10-day intervals. Spraying commenced when the plants were three weeks in the field and terminated about two weeks prior to harvest.

(*b*) *Method of Rating.*—(i) Overall rating. Immediately prior to the first harvest plots were rated on a scale ranging from 0 (=complete absence) to 5 (=average of 20 per cent. of leaf surface affected).

(ii) Detailed rating. About one week after the first harvest 20 plants in each datum row were given ratings for blue mould infection at three different levels, viz., ankle, knee, and hip high. The above scale was used. Results appear in Table 4.

Table 4.

EQUIVALENT MEAN RATING FOR BLUE MOULD DAMAGE IN 1953 FIELD TRIAL.

Treatment.	Overall.			Ankle.			Knee.			Hip.		
	Unprimed.	Primed.	Mean	Unprimed.	Primed.	Mean.	Unprimed.	Primed.	Mean.	Unprimed.	Primed.	Mean
Cuprous oxide	2·1	2·0	2·1	0·7	0·6	0·6	1·5	1·6	1·5	0·5	0·7	0·6
80% Ziram	2·4	2·5	2·4	1·3	1·0	1·1	1·6	1·6	1·6	0·7	0·5	0·6
Nil	1·7	1·7	1·7	2·0	1·7	1·9	1·1	1·2	1·1	0·5	0·5	0·5
Mean ..	2·1	2·0	2·0	1·3	1·1	1·2	1·4	1·5	1·4	0·6	0·6	0.6
	Nil << Ziram Nil < Cuprous oxide			Cuprous oxide << Nil Ziram < Nil			Nil << Cuprous oxide Nil << Ziram			No significant differences		
	No significant differences between primed and unprimed.											

Square root transformation used in analysis.

(4) Field Trial, 1954.

(*a*) *Treatments.*—Seven replications were made of three treatments:—home-made cuprous oxide (3 gal. stock–40 gal.); 65 per cent. Zineb (1½ lb.–40 gal.); and untreated.

Six applications were made at 7-day intervals. Spraying commenced when the plants were four weeks in the field and terminated after nine weeks in the field, about two weeks before harvest.

(*b*) *Method of Rating.*—(i) Overall rating. One week after spraying was terminated those plants which were flowering were rated on the scale used in the previous experiment.

(ii.) Detailed rating. One week later a maximum of 30 plants in each plot was examined. Top and bottom halves of these plants were rated separately. The same scale was used. Results appear in Table 5.

Table 5.

MEAN RATING FOR BLUE MOULD DAMAGE IN 1954 FIELD TRIAL.

Treatment.	Overall.	Bottom Half.	Top Half.
Cuprous oxide	1.4	2.06	1.8
65% Zineb	1.3	1.9	2.04
Nil	1.85	2.1	1.9

(5) Discussion of the 1952-1954 Field Trials.

These experiments demonstrated that the fungicides used were of little or no use for blue mould control in the field.

In 1951 a limited control was obtained. However, this trial was completed four weeks or more before harvesting commenced and subsequent experience has indicated that the position can change drastically in the period preceding harvest.

In 1952 early observations had indicated that the fungicides were giving some degree of control of the disease. However, a surprising situation was encountered when the plants were rated immediately prior to harvest and it was found that the intensity of infection on the unsprayed plants was significantly less than that on plants treated with either of the fungicides. The detailed readings subsequent to harvest revealed that, while the sprayed plants had less mould on the older foliage, the position was reversed on leaves higher up the plants.

In 1954 somewhat similar though less definite results were obtained. In this trial, although the overall ratings showed less mould on the sprayed plants, the detailed examinations later showed that while the amount of infection was less on the lower halves of sprayed plants than it was in this zone on unsprayed plants, it was greater on the upper halves of those plants sprayed with zineb than it was in a similar position on unsprayed plants.

While lack of control of the disease might be attributed to the inefficiency of the fungicides or of the schedules employed, one would not expect more intense infection on sprayed plants than on unsprayed plants.

Consideration of these data led to the adoption of the theory of acquired resistance, resulting from blue mould infection in the field. This matter will be discussed in more detail later.

(6) Field Trial, 1956.

Preventive spraying for blue mould control demands regular and efficient coverage of the surfaces of newly expanded foliage. Observations had indicated that one reason for the breakdown of control on sprayed plants in previous experiments was that such coverage was difficult to obtain because of the rapid growth of the tobacco plant. It was, therefore, decided to investigate the effects of more frequent applications and the 1956 trial was designed primarily for this purpose. The choice of fungicides was influenced by the results of the 1956 screening trials.

(*a*) *Treatments.*—Four replications were made of 8 treatments:—home-made cuprous oxide (3 gal. stock–40 gal.) plus white oil (1:320) plus malachite green (1:10,000); 65 per cent. zineb (1½ lb.–40 gal.); 90 per cent. ziram (4/5 lb.–40 gal.); and untreated.

Two schedules were used:—(i) One application per week; and (ii) two applications per week. Schedule 1 treatments received nine applications and

Schedule 2 treatments 18. Spraying commenced on Sept. 21, when the plants had been one week in the field, and was terminated on Nov. 20 just prior to harvest.

(*b*) *Method of Rating.*—On Nov. 22, subsequent to the final spray application, 10 plants in the datum row of each plot at or near the flowering stage were inspected and detailed recordings of blue mould incidence were made. The number of leaves on each plant was counted and each leaf was given a percentage rating for blue mould infection based on a visual estimate of the percentage of the lamina infected with the disease. The intensity of infection on each plant was gauged by calculating an average figure for percentage blue mould per leaf. The results are summarised in Tables 6 and 7. In Table 8 will be found particulars of the number of live leaves per plant.

Table 6.

Intensity of Blue Mould Infection in 1956 Field Trial.

——	Untreated.	Cuprous oxide.	Zineb.	Ziram.	Mean.
Untreated ..	9·3	..	..	..	9·3
Schedule 1	..	7·2	27·0	16·5	16·9
Schedule 2	..	2·0	10·3	8·2	6·8
Mean ..	9·3	4·6	18·7	12·4	11·9

Marginal differences:

Zineb >> untreated, cuprous oxide.
Zineb > ziram.
Ziram > cuprous oxide.
Schedule 1 > untreated, Schedule 2.

The variability was very high and not homogeneous. This was due to the infection pattern, which will be discussed below. When this factor is considered, the percentages taken over Blocks 3 and 4 only are better indications of the effects of the treatments. The relevant means for these blocks are given in Table 7.

Table 7.

Intensity of Blue Mould Infection in Blocks 3 and 4 of 1956 Field Trial.

——	Untreated.	Cuprous oxide.	Zineb.	Ziram.	Mean.
Untreated ..	14·2	..	..	..	14·2
Schedule 1	..	10·6	34·1	19·2	21·3
Schedule 2	..	2·4	10·1	11·3	7·9
Mean ..	14·2	6·5	22·1	15·2	14·5

Individual differences:

Cuprous oxide Schedule 2 << all others.
Zineb Schedule 1 >> all others.
Ziram Schedule 1 >> all except zineb.

Table 8.

Number of Live Leaves per Plant in 1956 Field Trial.

——	Untreated.	Cuprous oxide.	Zineb.	Ziram.	Mean.
Untreated ..	22·0	..	..	..	22·0
Schedule 1	..	24·9	21·4	23·4	23·2
Schedule 2	..	26·0	25·0	25·5	25·5
Mean ..	22·0	25·4	23·2	24·5	24·4

Marginal differences:
Cuprous oxide >> untreated.
Cuprous oxide > zineb.
Ziram > untreated.
Schedule 2 > Schedule 1, untreated.

Since the internal stem infection is such a serious phase of the disease, it was necessary to check the effect of treatment on the development of systemic mould. This was done by examining 25 plants in the datum row of each plot. With the aid of a knife the bark at the base of the stalk was raised and the presence or absence of the characteristic internal discoloration was noted. The percentage stem mould for the different treatments was as follows:—Schedule 1: cuprous oxide 4, zineb 1, ziram 1. Schedule 2: cuprous oxide 2, zineb 2, ziram 1. Untreated 44.

(*c*) *Discussion.*—By Nov. 5, three weeks after it had first appeared, the disease was prevalent but not serious in most of the unsprayed plots and was present to a minor degree in those plots treated with ziram and cuprous oxide. The incidence in the zineb-sprayed plots was negligible. On Nov. 13, one week after storm rain on Nov. 6, the disease was present in epiphytotic proportions in Blocks 3 and 4 and portions of Blocks 1 and 2. It was quite evident that those plots sprayed with zineb and ziram, particularly Schedule 1 treatments, were far worse affected than either those treated with cuprous oxide or the unsprayed plots. It was also quite evident that the infection was most intense on the middle leaves on these zineb-treated and ziram-treated plants. Later it extended to basal leaves (Figs. 2 and 3).

From the result presented above it can be seen that cuprous oxide plus white oil plus malachite green Schedule 2 was the only spray treatment which reduced blue mould infection to a significant degree. The plots treated once a week with zineb and ziram had a significantly greater intensity of infection than had the untreated plots.

These results were not entirely unexpected. The phenomenon of acquired resistance encountered previously in this work was undoubtedly responsible for the failure of the disease to intensify on the unsprayed plants. A somewhat similar experience has been described for another downy mildew type disease—downy mildew of cabbage (Cox 1957, p. 270), who found that "by harvest time the check plots were the 'cleanest'."

Fig. 2.

1956 Field Trial. Left-hand side: zineb once each week. Right-hand side: cuprous oxide once each week.

The inexplicable feature of the results reported here, however, was the extent of the breakdown in the protection afforded by dithiocarbamate sprays under epiphytotic conditions. Similar difficulties have been encountered in Victoria when nabam and zineb are used for tobacco blue mould control under conditions favourable to intense blue mould infection (R. G. Paddick, personal communication 1959).

When the figures for live leaves per plant listed in Table 8 are examined, it will be seen that particularly in the case of the Schedule 2 treatments there have been increases in the numbers of leaves on sprayed plants. The one treatment showing a decrease is zineb Schedule 1. It should also be noted that the incidence of stem mould has been reduced from an average of 44 per cent. in the unsprayed plots to an average of 2 per cent. in the sprayed plots.

It would, therefore, appear from the results of this experiment that early mould infections which confer a degree of immunity on the host also destroy valuable lower leaves and are associated with a high incidence of systemic mould.

(7) Field Trial, 1957.

The concentration of cuprous oxide employed in the 1956 trial gave rise to undesirable deposits on sprayed leaf, particularly when the mixture was applied twice each week. Spray burn was also a problem under certain conditions. In 1957 a weaker cuprous oxide mixture was used, viz., 1½ gal. stock to 40 gal. water. Other materials were introduced as a result of the latest screening trials. The following concentrations were used:—zineb 1½ lb.–40 gal.; chloranil 4 lb.—100 gal.; cottonseed oil (C.O.) ·75 per cent.; benzyl salicylate 1:1,600; malachite green (M.G.) 1:10,000.

Fig. 3.

1956 Field Trial. Left-hand side: zineb once each week. Right-hand side: unsprayed.

Since previous experience had shown that conditions suitable for the development of an epiphytotic did not usually occur before some six weeks after planting out, treatments commencing at planting time were compared with treatments commencing six weeks later. In three treatments (K, L, O) the plants were primed twice during the first six weeks.

The possibility that low-volume application of oil-based fungicides would be of use, particularly in the later stages of growth, prompted the inclusion

of early spraying plus late misting treatments. The machine used to apply the oily mists was a "Swingfog" with a misting attachment fitted. Cuprous oxide (a commercial preparation containing 80 per cent. copper) and zineb were each used at the rate of 1 lb. to 1 gal. oil.

(*a*) *Treatments.*—There were 3 replications of 16 treatments. Particulars of the latter are given in Table 9.

Table 9.

AVERAGE BLUE MOULD RATING FOR TOP AND CENTRE ZONE ON NOVEMBER 11, FIELD TRIAL, 1957.

Treatment.	Time of Application.	Mean Rating.	Significantly less than
K. Primed. Cuprous oxide + C.O.+ M.G.	late	0·8	(1%) P (5%) M, E, F
J. Benzyl salicylate + C.O.+ M.G. ..	late	1·2	(5%) F, P
B. Zineb + C.O.	early + late	1·3	(5%) P
D. Benzyl salicylate + C.O.+ M.G. ..	early + late	1·3	(5%) P
A. Cuprous oxide + C.O.+ M.G. ..	early + late	1·3	
G. Cuprous oxide + C.O.+ M.G. ..	late	1·3	
C. Chloranil + C.O.	early + late	1·5	
I. Chloranil + C.O.	late	1·5	
N. Zineb + C.O.	early	1·6	
Zineb mist	late		
L. Primed. Zineb + C.O.	late	1·7	
O. Primed. Nil spray		1·8	
H. Zineb + C.O.	late	1·9	
M. Cuprous oxide + C.O.+ M.G. ..	early	2·3	
Cuprous oxide mist	late		
E. Cuprous oxide + C.O.+ M.G. ..	early	2·4	
F. Zineb + C.O.	early	2·6	
P. Nil spray		2·7	

The experimental block was planted on Sept. 10. The initial sprays of the early treatments were applied on Sept. 17. The last spray applications in the early treatments (E, F, M and N) were made on Oct. 21 and 22. The first spray applications in the late spray treatments (G, H, I and J) were made on Oct. 28 and 29. Misting with oil-based fungicides (M and N) commenced on Oct. 29. Final applications of mists and sprays were made on Nov. 11 and 12. Thus the early spray treatments (E, F, M and N) were repeated six times at weekly intervals and the late spray treatments (G, H, I, J, K and L) four times. The plots which were sprayed each week from time of commencement (A, B, C and D) received 10 sprays.

(*b*) *Method of Rating.*—On Nov. 11, approximately nine weeks after planting out, a rating for blue mould incidence was made on 15 plants in each datum row. Each plant was rated in three positions. Counting downwards from the first leaf exceeding 9 in. in length, the foliage was divided into three

zones comprising the top five leaves, the centre five leaves, and the remaining bottom leaves. The following rating scale was used:—

0=Nil.

1=Scattered spots on one leaf; odd spots or nil on remainder.

2=Scattered spots on two leaves; odd spots or nil on remainder

3=Concentrated spots on one leaf; scattered spots on two leaves; odd spots or nil on remainder.

4=Concentrated spots on two leaves; scattered spots on remainder.

5=Concentrated spots on three leaves; scattered spots on remainder.

6=Concentrated spots on four leaves; scattered on one.

7=Concentrated spots on all leaves.

On Nov. 18, during the week following the final spray application, 10 plants at or near the flowering stage in the datum row of each plot were inspected and detailed readings on blue mould incidence were carried out. A leaf count was made on each plant commencing at and including the first leaf to exceed 15 in. in length. Estimates of percentage blue mould infection were given to each leaf as in the 1956 trial. The plants were again divided into three zones (top, centre and bottom), each zone in this case consisting of six leaves. Where leaves were killed they were given a rating of 100 per cent. blue mould infection. An average percentage mould infection figure was calculated per leaf for each zone.

Table 10.

Average Blue Mould Rating for Bottom Zone on November 11, Field Trial, 1957.

Treatment.	Time of Application.	Mean Rating.	Significantly less than
B. Zineb + C.O.	early + late	0	(1%) J, E, O, G, P (5%) H, I
N. Zineb + C.O.	early	0·1	(1%) J, E, O, G, P
Zineb mist	late		(5%) I
D. Benzyl salicylate + C.O.+ M.G. ..	early + late	0·2	(1%) J, E, O, G, P
A. Cuprous oxide + C.O.+ M.G. ..	early + late	0·4	
C. Chloranil + C.O.	early + late	0·5	
F. Zineb + C.O.	early	0·5	(1%) G, P
K. Primed. Cuprous oxide + C.O.+ M.G.	late	0·5	(5%) J, E, O
L. Primed. Zineb + C.O.	late	0·6	
M. Cuprous oxide + C.O.+ M.G. ..	early	1·0	
Cuprous oxide mist	late		(1 P %)
H. Zineb + C.O.	late	1·2	
I. Chloranil + CO..	late	1·2	
J. Benzyl salicylate + C.O.+M.G. ..	late	1·8	
E. Cuprous oxide + C.O.+ M.G. ..	early	1·8	(5%) P
O. Primed. Nil spray		1·8	
G. Cuprous oxide + C.O.+ M.G. ..	late	2·2	
P. Nil spray		3·2	

Results are given in Tables 9, 10 and 11. In Table 9 the figures are simplified by combining the ratings for top and centre as there was little difference between these regions at this time.

Table 11.

PERCENTAGE BLUE MOULD PER LEAF IN EACH OF THREE ZONES ON NOVEMBER 18, FIELD TRIAL, 1957.

Top.		Centre.		Bottom.	
Treatment.	Equiv. Mean %.	Treatment.	Equiv. Mean %.	Treatment.	Equiv. Mean %.
D	1·1	K	8·1	B	9·9
J	1·1	J	10·2	D	10·0
K	1·1	G	12·8	N	18·4
I	1·3	D	13·8	A	21·2
G	1·7	L	14·5	K	24·1
N	2·0	A	15·5	H	31·3
O	2·0	I	16·0	L	34·9
P	2·2	H	18·4	I	38·6
L	2·3	B	18·5	J	43·4
C	2·4	O	19·5	F	43·4
B	2·5	N	29·0	G	45·1
E	2·6	E	33·6	M	50·7
A	2·7	P	36·7	E	61·1
H	2·8	M	44·2	C	62·8
F	4·5	C	46·0	O	70·8
M	4·9	F	50·7	P	85·7
D, J, K < F, M I < M		K << C, F ; < E, P, M J << F ; < P, M, C G, D, L < M, C, F A, I < C, F H, B, O < F		B, D << O, P ; < E, O N, A, K << P ; < O H, L, I < P	

Prior to analysis percentages were transformed by means of the inverse sine transformation.

(*c*) *Discussion.*—Until Oct. 22 blue mould infection was scattered in the experimental area with the exception of Block 3, where there had been a rapid spread. In this block at this date the pattern was much as would be expected with those plots receiving early spray treatments showing less disease than the unsprayed plots. An interesting feature at this time was the reduced amount of blue mould in treatments K, L, and O, which had been primed once at this juncture.

By Nov. 11, subsequent to a rain group which commenced on Oct. 30 and lasted until Nov. 6, all plants in the three blocks were infected. The intensity of infection was increasing rapidly in Blocks 1 and 2. In Block 3, however, due to the effect of acquired resistance, the severity of infection did not show a substantial increase. At this date it was plain that the treatments which involved weekly sprays from the time of commencement (A, B, C and D)

had considerably reduced blue mould damage on the lower leaves. Treatments M and N also had less infection in this zone. The late-sprayed treatments commenced on Oct. 28 prior to the beginning of the rain group and consequently treatments H, I, J, K and L conferred a degree of protection on lower leaves. Spraying for the first six weeks only produced no reduction of mould in the upper zone of foliage.

Between Nov. 11 and Nov. 18, when the final assessments of foliage damage in the various treatments were made, the intensity of infection reached a maximum. In the bottom zone the benefits of spraying were plainly shown by the reduced incidence of mould on the lug leaves in the best treatments. There was little to choose between the zineb, benzyl salicylate and cuprous oxide sprays in this respect. Zineb applied as an oily mist also appeared to be effective but its use is accompanied by the risk of serious leaf burn.

For the top and central zones the main features shown were—

(i) The improvement in the position of the unsprayed and the late-sprayed treatments (treatments L and H excepted). This was no doubt due to the acquired resistance factor, which could have operated to the advantage of all these treatments, particularly those in Block 3, which had been exposed to infection for six weeks before treatment commenced.

(ii) The deterioration in the position of treatments B (zineb) and A (cuprous oxide), which was reminiscent on a much smaller scale of the breakdown of zineb in the 1956 trial when inoculum potential was high. The anomalous position of treatments L and H mentioned above could also have been due to this breakdown. Treatment C (chloranil) gave reasonable control of the disease until an epiphytotic developed, when it also lost its efficiency. The superiority of treatment I over treatment C might well be linked with the acquired resistance factor.

(8) Field Trial, 1958.

Screening trials had demonstrated the usefulness of zineb-salicylate mixtures (Table 2), and accordingly a zineb-methyl salicylate spray was chosen for use in the 1958 field trial. The composition of this spray was:—zineb 1½ lb.–40 gal. + cottonseed oil 0·75 per cent. + methyl salicylate (M.S.) 1 lb.–27 gal. Zineb plus a wetting agent (Triton B1956) was included so that the behaviour of the two mixtures could be compared if an epiphytotic of the disease occurred. In treatment G the seedlings were dipped in the zineb-methyl salicylate mixture before planting out.

(*a*) *Treatments.*—There were three replications of eight treatments, particulars of which appear in Table 12.

The experimental area was planted on Sept. 11. Spray applications commenced on Sept. 29. The late-spray treatments B and D commenced on Nov. 3. The final applications were made on Nov. 17. The plots sprayed twice

each week from time of commencement received 15 applications; those that were sprayed once each week from that time received eight applications; and the late-sprayed treatments received three applications.

Table 12.

EQUIVALENT MEAN PERCENTAGE BLUE MOULD PER LEAF AND EQUIVALENT MEAN STEM MOULD PER TREATMENT IN 1958 FIELD TRIAL.

Treatment.	Application.	Nov. 7.	Nov. 18.	Nov. 18.			Stem Mould.
				Top.	Centre.	Bottom.	
A. Primed. Nil spray ..		14·2	4·7	0	0·4	11·9	26·7
B. Primed. Zineb + C.O. + M.S.	late only	12·9	3·9	0	0·3	9·1	19·6
C. Nil spray		14·1	5·7	0	0·6	15·4	24·0
D. Zineb + C.O.+ M.S. ..	late only	14·8	2·4	0	0·8	7·5	20·3
E. Zineb + C.O.+ M.S. ..	weekly	4·4	2·9	0	0·3	8·6	2·5
F. Zineb + C.O.+ M.S. ..	twice each week	2·0	1·4	0	0·8	3·2	1·3
G. Zineb + C.O.+ M.S. (seedling dip)	weekly	5·0	2·7	0	0·3	9·1	2·9
H. Zineb + Triton B1956 ..	weekly	8·3	4·1	0·3	0·8	11·9	16·5
		E, F, G << A–D F << E, G, H E < H < D	No sig. diff.	Observed means. Not analysed		No sig. diff.	E, F, G << A–D, H

Analysis in terms of log X.

(*b*) *Method of Rating.*—On Nov. 7, soon after commencing the late-spray schedule, all the plants in the datum row of each plot (usually 35) were inspected and leaf counts were made which included all leaves exceeding 9 in. in length. Each leaf was given a percentage rating for blue mould damage as in previous trials and a mean figure was calculated as before.

On Nov. 17 and 18, similar readings were made on 10 plants at the flowering stage in each datum row. The same method of disease assessment was used and a mean percentage infection figure was calculated. In addition, the plants were divided into three zones of foliage, each consisting of six leaves, and mean percentage infections per leaf were calculated for each zone. Dead leaves were regarded as 100 per cent. infected.

At intervals from Nov. 18 to Dec. 15, all the plants in this trial were examined for stem infection. A mean figure for percentage stem mould was calculated for each treatment.

(*c*) *Discussion.*—During the course of this experiment a serious outbreak of blue mould failed to eventuate, so the fungicides and schedules used were not subjected to the harsh test of an epiphytotic as had occurred in previous years.

Practically 100 per cent. of the plant population was infected with the disease on Oct. 31, but to a minor degree only. The intensity of infection had probably reached a peak on Nov. 7, when the average percentage infection per leaf for the worst affected treatment was 15·6. After this date, fresh infection was at a minimum, due perhaps to the combined effects of acquired resistance and unsuitable weather conditions.

At Nov. 7 it was too soon to assess the value of the late-spray treatments B and D, for these had received their first spray application only on Nov. 3. All the other zineb treatments had effectively reduced blue mould. Priming at four and seven weeks after planting out (A and B) gave no benefit in this trial.

On Nov. 18, as a result of the factors mentioned above, the overall percentage infection was low and the differences between treatments not significant. There is a suggestion that late spraying had some effect.

There was a certain amount of phytotoxicity associated with the use of the zineb plus cottonseed oil plus methyl salicylate mixture. This was particularly noticeable on those plants sprayed twice each week. The symptoms appeared on the lowest leaves and varied from an abnormal chlorosis (tending towards a bronzing) to necrotic patches of variable size. Bottom leaf from plants which had been sprayed repeatedly with this mixture also did not cure satisfactorily.

This trial confirmed the results obtained in screening trials which demonstrated the superiority of the zineb plus cottonseed oil plus methyl salicylate mixture over zineb alone for blue mould control. In the absence of suitable conditions, it was not possible to state whether this spray possesses the major disadvantage of zineb alone—i.e., the breakdown in efficiency which occurs when conditions are very favourable for disease development. In addition, the adverse effect of the mixture on curing excludes any possibility of its recommendation to growers until further work has been done on formulation and method of application.

(9) GENERAL CONCLUSIONS FROM THE FIELD TRIALS.

Early trials gave little indication that control of blue mould by any of the fungicides used was possible under field conditions. The 1956 trial, however, showed that the addition of white oil and malachite green to cuprous oxide improved the performance of this fungicide and the disease was controlled by applications twice each week. The benefits of spraying were rendered less obvious by low blue mould readings on unsprayed plants due to acquired resistance.

In the 1957 trial the plants were once again subjected to an epiphytotic. The most successful treatment, taking into consideration the whole growing period, was benzyl salicylate plus cottonseed oil plus malachite green (D, J). Some phytotoxicity problems associated with this mixture are yet to be overcome.

Treatments involving zineb and cottonseed oil (B, N) were very effective in reducing mould on the lower leaves under conditions of moderate infection but lost efficiency when infection intensified. The reason for the breakdown at a critical time shown by the dithiocarbamate sprays is obscure. An attempt to overcome this in the 1958 trial by the addition of methyl salicylate and by spraying at more frequent intervals failed to yield an answer, as the usual late epiphytotic did not eventuate.

Regarding schedules, the most successful in the 1957 trial involved applications once each week from one week after planting out until immediately prior to harvest. Late-spray schedules initiated prior to the onset of wet weather proved of more benefit than early schedules which terminated at this time, but the acquired resistance effect probably operated to the advantage of the late-schedule sprays.

The control of mould on the lower leaves during the early part of the season is associated with a marked reduction in the amount of basal stem mould. Some of the zineb combinations are quite effective in this respect.

In summing up the results of these field trials it is apparent that a blue mould fungicide which can be recommended without reservation to tobacco growers is not yet available. However, mixtures have been tested which have improved on the performances of standard fungicides. It is also plain that successful spray treatments are less likely to result in spectacular reductions in leaf mould infection than in less obvious but equally important reductions in the incidence of systemic infection.

V. ACQUIRED OR INDUCED RESISTANCE TO TOBACCO BLUE MOULD.

Acquired immunity to certain plant virus diseases is a well-known reaction of the host (Price 1940) and has been used as a control measure in some cases. Acquired immunity or resistance of a plant to a fungus disease is not a common occurrence, however. The subject has been reviewed in some detail by Chester (1933).

The resistance of tobacco seedlings, which have been infected with tobacco blue mould and recovered, to further attack by the fungus has been described (Wolf 1939, Clayton *et al.* 1938) and it has been noted that, when infected tobacco seedlings are transferred to the field and stem infection develops, leaf-spotting on such plants is seldom as pronounced as that on plants from healthy seedbeds (Angell 1957).

Earlier in this paper a theory of acquired resistance to blue mould infection was adopted in order that the results of certain early experiments could be interpreted. This theory received support after an independent authority had studied the data from these early experiments (G. S. Taylor, personal communication 1957).

It is possible that the results obtained in Florida (Cox 1957) with a related disease, downy mildew of cabbage, previously commented on, were due to the operation of a similar effect.

(1) Discussion of Evidence Provided by Field Trials.

The results of the 1956 field spray trial were expressed graphically by plotting the average percentage blue mould infection for a leaf against the serial number of that leaf (Fig. 4). The number of leaves used for comparison

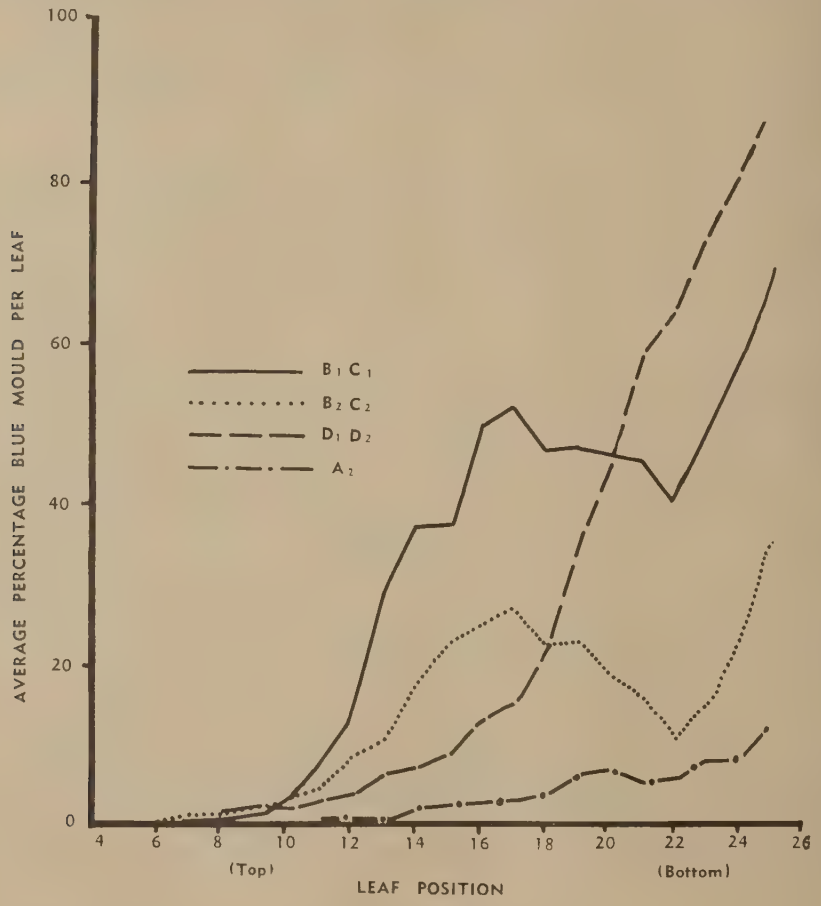

Fig. 4.

Resistance to Blue Mould in Relation to Fungicidal Treatment. 1956 Field Trial. A_2 — Cuprous oxide + white oil + malachite green, twice a week. B_1, C_1 — Mean zineb and ziram plots, twice a week. B_2, C_2 — Mean zineb and ziram plots, once a week. D_1, D_2 — Mean unsprayed plots.

purposes was 25, the average number of leaves for the best spray treatment. These were numbered from 1 (top) to 25 (bottom). Dead leaves were given values of 100 per cent. blue mould infection.

A mean of the percentage blue mould figures for the four replications was taken and in order to obtain representative examples of different spray effects the following selections or combinations of treatments were used—cuprous oxide + white oil + malachite green, twice each week (A_2), representing an efficient fungicide; a combination of the figures for zineb and ziram weekly (B_1, C_1) and twice each week (B_2, C_2), representing a fungicide liable to break down under stress; and a combination of the two nil treatments (D_1, D_2).

If the acquired resistance theory were to be substantiated, the following types of curves would be expected:—

(*a*) The curve for unsprayed treatments. This should be quite steep, indicating infection and death of lower leaves with subsequent reduced infection on upper leaves due to resistance. This is well illustrated in Fig. 4.

(*b*) The curves for those treatments which were ineffective when the inoculum potential was at a maximum. These would be expected to commence their final steep drop much further towards the left, indicating the disposition of susceptible foliage higher on the plant due to the protection afforded by these sprays earlier and the consequent lack of acquired resistance. The location of the maxima would be fixed by the position on the plants of the most intense infection. In Fig. 4 the zineb-ziram curves for both weekly and twice-each-week applications have been included. These both conform to the anticipated general shape with the twice-each-week exhibiting the less extensive infection.

(*c*) The curve for an efficient treatment. This should be quite flat, showing little increase with increase in position number, indicating low mould incidence on all leaves. This is well illustrated by the curve for the twice-each-week cuprous oxide spray.

In considering the effect of acquired resistance in the 1957 spray trial, the unsprayed plots only have been selected to illustrate the point. In this experiment blue mould was widespread in Block 3 prior to Oct. 22 but was virtually absent from Blocks 1 and 2. Apparently due to the acquired resistance the unsprayed plots in Block 3 showed much less injury than those in the other two blocks on Nov. 18, i.e., subsequent to the November epiphytotic. The actual state of affairs is shown in Tables 13 and 14.

Table 13.

BLUE MOULD RATING IN UNSPRAYED PLOTS ON OCTOBER 22, FIELD TRIAL, 1957.

Treatment.	Block 1.		Block 2.		Block 3.	
	Bottom.	Top.	Bottom.	Top.	Bottom.	Top.
O ..	·6	·1	·2	·1	1·9	0·8
P ..	·1	0	·2	0	3·1	1·2

Table 14.

AVERAGE PERCENTAGE OF BLUE MOULD PER LEAF IN EACH OF THREE ZONES ON NOVEMBER 18, FIELD TRIAL, 1958.

Treatment.	Block 1.			Block 2.			Block 3.		
	Bottom.	Centre.	Top.	Bottom.	Centre.	Top.	Bottom.	Centre.	Top.
O ..	60·4	13·5	2·3	80·3	26·0	1·8	25·7	1·7	0·4
P ..	92·9	47·1	3·4	76·4	27·0	1·2	9·7	2·5	0·2

At the Oct. 22 examination each plant was divided for the purpose of rating into two approximately equal zones from the uppermost leaf exceeding 9 in. in length downwards. The rating was on a scale from 0 (nil) to 5 (chlorosis or concentrated spotting on all leaves).

On Nov. 18 the examination and rating was made as described for the Field Trial, 1957.

The results of the disease assessments made on Nov. 18 on the unsprayed plots are shown in graph form in Fig. 5. As in Fig 4 this was done by plotting the average percentage blue mould per leaf against the position number of the leaves.

Once again the steep curves typical of non-sprayed plants with acquired resistance were the result. However, the protection provided by the early infection in Block 3 is made quite apparent by the position of the curve for this Block.

In 1958 a trial was designed to ascertain whether a resistance to blue mould could be induced by a deliberate early infection with the disease and, if so, whether any practical importance could be attached to it.

In one treatment seedlings were infected at an early age in the seedbed. Eradication was then attempted with benzol fumigation subsequent to the appearance of sporing blue mould lesions but was not successful. Large numbers of inoculated seedlings developed vascular infection with consequent high mortality both in the beds and on transfer to the field.

In another treatment the seedlings were inoculated a few days prior to transplanting and had not developed obvious mould lesions when they were shifted. In this treatment, too, a large percentage of the plants were infected with stem mould and many died.

Conditions in 1958 were not conducive to field infection and it was impossible to detect differences between any of the treatments. However, the data did demonstrate a relationship between systemic infection and reduced intensity of leaf infection (Table 15).

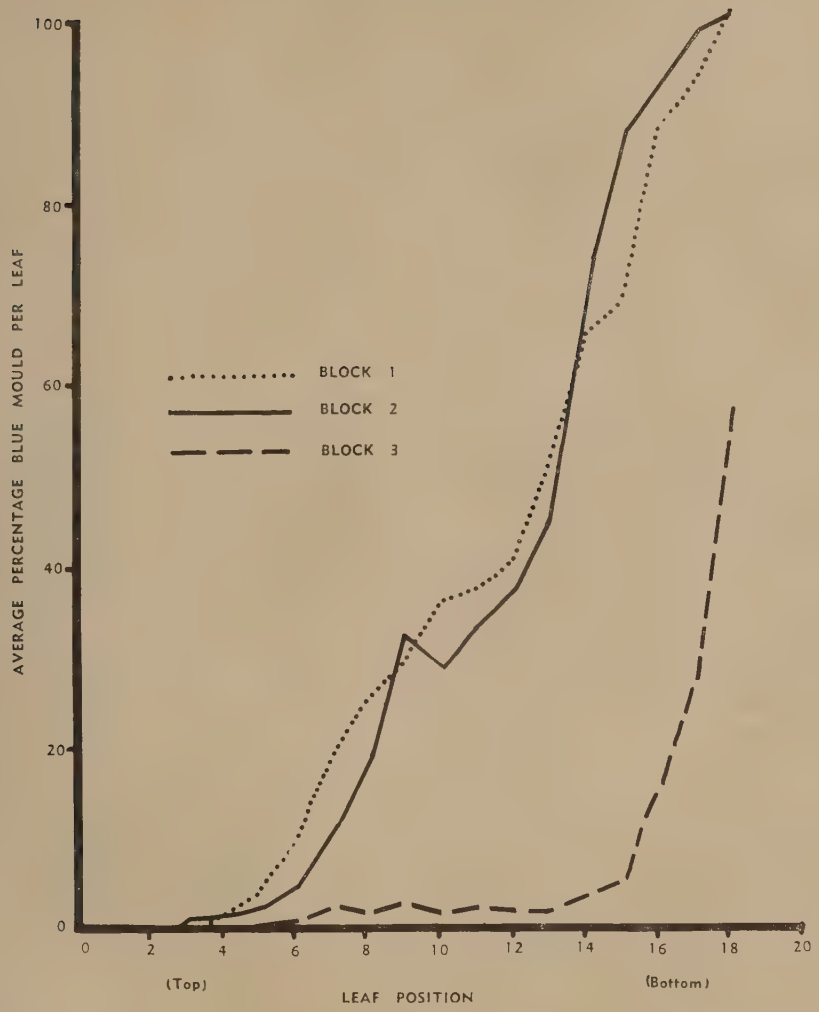

Fig. 5.

Effect of Early Blue Mould Infection on Subsequent Mould Development. 1957 Field Trial. Blocks 1 and 2, late infection. Block 3, early infection.

Under the conditions of this experiment and in the absence of an epiphytotic there was no evidence to presume that any infection other than a systemic one induces a resistance to subsequent leaf infection. This is not surprising, for it has been said (Butler and Jones 1949) that "it is remarkable fact that the only cases in which it has been fully demonstrated that infection

can lead in plants, as in animals, to immunity from subsequent re-infection occur in the most completely systemic plant diseases that are known, those due to viruses.''

Table 15.

Association Between Stem Mould and Leaf Mould in 1958 Acquired Resistance Trial.

Plants without Stem Mould.		Plants with Stem Mould.	
Average No. of Leaves.	Average Percentage Blue Mould.	Average No. of Leaves.	Average Percentage Blue Mould.
13·3	4·8	12·1	0·5

(2) Conclusions.

The evidence put forward here suggests that tobacco plants, when exposed to blue mould infection, acquire a resistance to subsequent re-infection. However, this resistance is coupled with systemic infection which in seedlings may lead to stunting and death. On field plants the results of systemic infection are often not so obvious but lug leaf is destroyed and weakened stalks usually result with consequent danger of lodging before the crop is harvested. It is unlikely therefore that advantage can be taken of this phenomenon for reducing loss from the disease.

It is, however, necessary to take into account the possibility of acquired resistance when interpreting routine fungicide trials in order to explain what otherwise appear as anomalous results.

VI. ACKNOWLEDGEMENTS.

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DDT FOR THE PROTECTION OF STORED POTATOES.

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SUMMARY.

At the strengths used, DDT, derris and pyrethrum-piperonyl butoxide as dusts and DDT-impregnated Chapman sacks and sugar-bags were all efficacious in protecting stored tubers when the initial infestation was less than 4 per cent. DDT dusts, ranging in concentration from 0.5 to 2 per cent., and containers treated in DDT emulsions ranging in concentration from 0.5 to 2.5 per cent. arrested infestations varying from 8 to 35 per cent. satisfactorily. The higher levels of DDT were more efficacious, however, when the initial infestation exceeded 20 per cent.

Results with pieces of DDT-impregnated burlap and DDT-treated paper strips placed among the tubers were not conclusive.

A 2 per cent. DDT dust applied at the rate of ½ lb. per bag, and a dipping strength of 2 per cent. DDT emulsion for impregnating containers, were considered the most efficacious for general application in the field.

Second-hand lightweight hessian sugar-bags provide a satisfactory alternative to the standard Chapman sacks for storing tubers. Multiple-layered paper bags are not suitable for storing tubers under Queensland conditions.

Quantities of DDT are transferred to tubers when DDT-impregnated containers are handled. DDT residues on tubers, whether acquired from the container or from dust application, persist during storage but are reduced to within safe limits during handling from store and preparation for the table.

I. INTRODUCTION.

Potatoes from the spring-planted crop in South Queensland are harvested in early summer and provide tubers for table use and seed for planting the autumn crop. Also, tubers produced during the winter months in more northern parts of the State are harvested when temperatures are rising. A considerable proportion of these tubers may be stored for a long period under conditions suited to the rapid development of the potato tuber moth, *Gnorimoschema operculella* (Zell.). The incidence of the pest in the tubers from these crops at harvest and during storage is therefore of major importance.

Field control of the tuber moth in Queensland is designed chiefly to provide maximum yields of sound tubers of marketable size (May 1952). At harvest, some infested tubers are often inadvertently included with sound tubers during bagging operations in the field. Also, in bulk stores, where levels of moth populations may be high during summer, infestation from outside the bags is an added problem. The need to destroy these nuclei of infestation from the field and to prevent reinfestation during storage has always been appreciated in Queensland.

The problem of protecting stored tubers from *Gnorimoschema* attack is not confined to this State. Derris, applied as a dust to bagged tubers, was generally recommended in Australia prior to 1940, but its unavailability in later years induced a search for alternative materials. Many inert dusts, including magnesite, pyridine, ferric oxide and kaolin, were tested with varying

Fig. 1.

Larval Damage to Tubers. Lower tuber not infested.

success (Helson 1942; Lloyd 1943). These materials were merely protectants and for success required humidities below 80 per cent. and a very low initial incidence of infestation (Helson 1942).

Lloyd (1947) advocated dusting tubers with derris soon after fumigation with carbon bisulphide. Fumigation is not practicable under the system of

handling adopted in Queensland, and application of an effective insecticide during bagging operations in the field is a more suitable method of control. Walker and Anderson (1944) reported good tuber moth control in seed potatoes with 2 per cent. DDT dust.

Following tests in North Queensland with DDT, derris and magnesite, and in South Queensland with derris, magnesite and other inert dusts (Brimblecombe and Cannon 1949), it was concluded that either DDT or derris could be advocated for protecting stored table potatoes. Helson (1949) had also shown that the addition of ferric oxide did not impair the protection afforded by DDT dust and left tubers a desirable colour.

The dependence of inert dusts on humidity and a low initial infestation for the successful protection of stored potatoes, and the limited availability of derris dust, meant these materials were unsatisfactory for general recommendation in Queensland. The application of a brown-pigmented 2 per cent. DDT dust, at the rate of 8 oz. per bag, as the tubers were bagged for market was considered a more practical method of control.

Though the efficacy of DDT dust, when properly applied, had been demonstrated, further testing at various levels of initial infestation and reinfestation was necessary, while the status of residues on DDT-dusted table potatoes at the end of storage warranted investigation.

In contrast to direct application of insecticidal dust, impregnating the container with DDT had been employed by Hayhurst (1945) and Parkin (1948) to protect stored grain from insect attack. In America, Hofmaster and Anderson (1948) had prevented tuber infestation by storing clean tubers in DDT-impregnated bags, while confetti and paper streamers treated with 5 per cent. DDT significantly reduced tuber infestation but did not give complete control. These workers found that moths emerging in DDT-treated containers moved freely among the tubers and were attracted to the light filtering through the sacking. These moths died within 24 hours of emergence. This American work and somewhat similar tests by Lloyd (1951) in New South Wales were conducted under laboratory conditions.

These methods of applying DDT to protect stored tubers might, it was thought, overcome some of the supposed problems associated with direct DDT dust application and warranted study under field and commercial conditions.

Experiments were carried out therefore in the Gatton district, Lockyer Valley, for three consecutive seasons, commencing in the summer of 1949-50, to investigate the relevant aspects of *Gnorimoschema* control in stored tubers. This involved studying the effects of levels of DDT applied as direct dusts or impregnated in the storage containers, of which three types were used, and of placing strips of DDT-impregnated burlap or brown paper among the tubers. Alternative dusts of possible control value were compared with the current standard. The persistence of the DDT residues in containers and on tubers during storage was also investigated.

II. TUBER PROTECTION.

(1) Materials.

(a) Potatoes.

The variety Sebago was used throughout this work. All tubers were first and second grades as bagged on the farm for marketing. Percentages of tuber moth infestations in the bulk samples varied with source, depending on control measures used during the growth of the crop, but all were within the limits of commercial supply. Normally, tubers are treated with insecticide during bagging operations. The potatoes for these experiments were removed from the field and sampled for tuber moth infestation before insecticides were applied. In some instances, a short time elapsed before this sampling could be undertaken.

Before commencing each experiment, damaged and misshapen tubers were discarded. Where uninfected tubers were required, those showing evidence of larval tunnelling were also discarded. Some, however, usually escaped the sorters, as it was impracticable to identify a proportion of those carrying only eggs and newly emerged larvae.

(b) Containers.

The three types used were:—

(1) The Chapman sack or Australian standard cornsack—41 in. x 23 in. hemmed, 2¼ lb., 8 porter, 9 shot, plain dry sewn.

(2) 70 lb. sugar-bag—manufactured by Colonial Sugar Refining Co. Ltd., Sydney, from 12 oz. hessian, 12 porter, 15 shot.

(3) Paper bag—a 5-layered, heavy-duty brown-paper bag.

Only the paper bags were new. The sugar-bags were once-used, while the Chapman sacks had been discarded for grain storage and were representative of bags normally used for potato marketing. With the exception of the experiment concerned with comparing storage containers, the Chapman sack was used throughout this work.

(c) Insecticides.

The following insecticides were used:—

DDT emulsion.—An emulsion concentrate containing 25 per cent. *p.p'* isomer, w/v: formulated with 70 per cent. *p.p'* isomer DDT.

DDT dispersible powder containing 40 per cent. *p.p'* isomer: formulated with DDT containing 80 per cent. *p.p'* isomer.

DDT dust containing 2 per cent. *p.p'* isomer in pyrophyllite.

Derris dust containing 0·75 per cent. rotenone.

Pyrethrum-piperonyl butoxide dusts.—

(*a*) Piperonyl butoxide 0·8 per cent., pyrethrins 0·05 per cent., in a carrier of exhausted pyrethrum powder 20 per cent. and talc 80 per cent.

(*b*) Piperonyl butoxide 1·6 per cent., pyrethrins 0·1 per cent., in a carrier of exhausted pyrethrum 20 per cent. and talc 80 per cent.

Kaolin.—A diluent, made from a Queensland clay.

The various treatments are detailed in the tables of results.

(2) Storage Conditions.

The insecticide screening experiment was conducted during 1951-52 in a well-ventilated basement of a concrete building; the other five were located in a 64 ft. x 20 ft. room constructed for storing potatoes below ground level.

During the 1949-50 series of experiments, temperatures near floor level during the months of December and January were 76 ± 4 deg. F.; in the same period, screen temperatures ranged between 63·7 deg. F. and 95·1 deg. F. Under these favourable conditions frequent inspections and sorting to remove rotten tubers were usually unnecessary, and thus comparisons of the effects of treatments on tubers were possible.

(3) Methods.

(a) Dust Application.

All dusts were applied at the rate of 8 oz. per bag for Chapman sacks and 4 oz. per bag for sugar-bags and paper bags. The dust required for treating each bag of tubers was divided into 1 oz. portions. One was placed in the bottom of a 4 gal. drum, which was then filled, and a further portion was poured over the tubers before these were tipped into the bag. This process was repeated until each bag was filled. Subsequent handling into store ensured a further spreading of dust among the tubers.

(b) DDT Impregnation.

Strengths of dips, prepared from both emulsion and dispersible powder, ranged from 0·5 per cent. to 5 per cent. Each jute container was treated separately and after immersion and thorough wetting was passed through a mangle at a fixed setting. Sacks were folded longitudinally, but sugar-bags readily passed through without folding. After thorough air-drying the containers were bundled according to type and treatment.

Paper bags were treated by pouring 1½–2 pints of dipping fluid into each bag and shaking to ensure complete wetting of the inner surface. After pouring out the surplus, the bag was held open with a light wooden frame until dry.

Burlap strips were made by cutting a Chapman sack impregnated with 2 per cent. DDT emulsion into eight equal portions. Four of these were placed amongst the tubers in each sack.

Brown-paper streamers ½ in. wide, were dipped in 5 per cent. DDT emulsion and dried, and 3 oz. of this treated paper was scattered amongst the tubers in each sack.

Randomised layouts were used with 4–6 replicates, depending on the number of treatments and the availability of suitable tubers. The plot unit was one sack or bag containing approximately 500 tubers in each Chapman sack and half this number in the other containers. The relevant details including layout are given in Tables 1 and 2.

All bags comprising an experiment were placed together on end in a single layer. Broad wooden slabs spaced beneath the bags and passages between replicates as well as between experiments ensured adequate ventilation. Once in position, bags were removed only for sampling purposes and then returned to the original positions. Bulk potatoes in another part of the store room and untreated plots within each experiment provided a continuous source of *Gnorimoschema* infestation throughout storage.

(c) Duration of Storage.

The period of storage depended on tuber decomposition and the purposes of the experiment. The method of handling varied also for each experiment, some bags being sampled for tuber infestation during storage (secondary inspections). A summary of relevant details is given in Table 1.

Table 1.

DETAILS OF STORAGE.

Experiment.	Commencement of Storage.	Duration (weeks).	No. of Secondary Inspections.
I	Dec. 12, 1951	13	2
II	Dec. 8, 1949	9	..
III (a)	Nov. 17, 1949	9	..
(b)	Nov. 18, 1949	9	..
(c)	Dec. 29, 1950	10	1
IV	Dec. 5, 1950	15	1
V	Jan. 4, 1951	9	1
VI	Jan. 5, 1951	6	..

(4) Assessing Results.

(a) Tuber Infestation.

Throughout these experiments, *Gnorimoschema* activity was determined as percentage of tubers infested. Many earlier workers (Helson 1942; Lloyd 1943; Hofmaster 1949; Lloyd 1951) had used moths, eggs laid and larval populations as criteria to determine efficacy of insecticide treatment. These workers had experimented with small containers in the laboratory but such detail could not be applied satisfactorily in these experiments. Without dissecting tubers, the old and fresh larval tunnelling could not be differentiated, nor could larval density per tuber be considered.

Moths, both dead and alive, and pupae were often encountered when bags were sampled for tuber infestation, but tuber breakdown, dust residue, dirt and other foreign material in the bags interfered with attempts at accurate determinations of moth populations. Percentage tuber infestation, however, was found quite adequate for the purposes of these studies.

Infestation per plot was determined by examining a number of samples each of 10 tubers taken at random from each bag. The number of samples taken differed with the experiment and was determined by the number of replicates (see Appendix I). The relevant information for each experiment is given in Table 2.

Table 2.

SAMPLING PROCEDURE.

Experiment.	Number of Replicates.	Number of Samples per Plot.	Total Tubers Examined per Treatment.
I	4	10	400
II	5	8	400
III (a) ..	6	8	480
(b) ..	6	8	480
(c) ..	4	12	480
IV	5	8	400
V	4	12	480
VI*	4	8	320

* Containers were half the capacity of those used in all other experiments.

The incidence of tuber moth was determined at the commencement, conclusion and in some instances (secondary inspections, see Table 1) during the period of storage. With the exception of Experiment II, samples were selected at random from each plot as the containers were being filled or after the tubers had been tipped from the container at the conclusion of each experiment. When Experiment II terminated, samples were taken from the upper, middle and lower portions of each sack as tubers were removed individually. From each portion, tubers were selected that were either in contact with the sack or from the centre.

At the final sampling in each experiment, tubers were washed to facilitate examination.

(b) Tuber Breakdown.

No mechanically damaged or obviously unsound potatoes were included when bags were filled at the commencement of each experiment. By recording the number of sound tubers at the commencement and the end of each experiment, the percentages of rotted tubers per bag were determined.

(5) Results.

(a) Experiment I—Screening of Insecticides.

At each secondary sampling, all unsound potatoes were discarded before re-bagging to improve storage. Results are given in Table 3, and representative tubers are shown in Fig. 2.

Fig. 2.

Comparisons Between Tubers After 13 Weeks' Storage (Experiment I). From top downwards:—pyrethrum-piperonyl butoxide dust (a); derris; derris (washed); DDT-treated sack; DDT dust (not pigmented); control. (Note infestation in control.)

Table 3.

EXPERIMENT I: TUBER MOTH INFESTATION AND BREAKDOWN.

Treatment.	Sampling Dates.				Percentage Tuber Breakdown.
	Dec. 12, 1951.	Jan. 30, 1952.	Feb. 21, 1952.	Mar. 17, 1952.	
	Percentage Tubers Infested.				
1. DDT-treated sack (2·0% emulsion)	4·2	3·5	1·5	1·0	35·4
2. DDT 2·0% dust	2·0	2·5	0·8	0·0	41·6
3. Derris dust	2·0	3·0	1·5	1·2	29·7
4. Pyrethrum-piperonyl butoxide dust (a)	2·8	3·5	1·5	1·5	37·2
5. Pyrethrum-piperonyl butoxide dust (b)	2·0	4·5	2·5	0·2	44·7
6. Kaolin	3·8	5·8	4·5	6·0	32·6
7. Control	3·0	13·0	37·5	66·5	73·4
Differences necessary for significance { 5%		2·4	1·9	1·4	
1%		3·3	2·6	1·9	

The insecticidal dusts were more efficacious than kaolin in preventing moth infestation. Tuber breakdown occurred in all treatments but was far more pronounced in the controls. Differences between treatments, however, were not significant.

(b) Experiment II—Preventing Infestation.

This experiment, using sound tubers, was run in two sections: in the first, four concentrations of dust were used, and in the second, the containers were impregnated with three levels of DDT emulsion.

Table 4.

EXPERIMENT II: PREVENTION OF INFESTATION.

Treatment.	Percentage Tubers Infested.		
	Centre of Sack.	Away from Centre of Sack.	Mean Percentage Infestation.
1. DDT 2·0% dust	5·0	4·0	4·8
2. DDT 1·5% dust	3·0	3·0	3·0
3. DDT 1·0% dust	3 0	4·5	3·8
4. DDT 0·5% dust	7·0	7·5	7·2
5. DDT-treated sack (2·5% emulsion)	0·0	2·0	1·0
6. DDT-treated sack (1·0% emulsion)	1·5	2·0	1·8
7. DDT-treated sack (0·5% emulsion)	3·0	3·5	3·2
8. Control	36·2	34·5	35·4
Differences necessary for significance .. { 5%			3·2
1%			4·2

The sections were combined for analysis as all potatoes were from the same source, and infestations in the controls were of the same order. Results are given in Table 4.

No relationship existed between infestation and position of tubers in the sack. The results of this experiment, however, indicate a range of DDT levels giving satisfactory protection.

(c) Experiment III—Arresting Infestation.

Concurrent with Experiment II, the same treatments were compared for arresting infestation already well established in the tubers at the commencement of storage (Experiments III (a) and (b)). The results are given in Tables 5 and 6.

Table 5.

Experiment III (a): Arresting Infestation.

Treatment.	Percentage Tubers Infested.		
	Commencement of Storage.	End of Storage.	Increase.
1. DDT 2·0% dust	27·1	38·5	11·5
2. DDT 1·5% dust	20·6	43·1	22·5
3. DDT 1·0% dust	22·1	42·1	20·0
4. DDT 0·5% dust	18·8	41·7	22·9
5. Control	22·7	90·8	68·1
Differences necessary for significance 5%	6·0	7·2	7·1
Differences necessary for significance 1%	8·2	9·8	9·6

Experiment III (c) covered both sack and dust treatments as in Experiment III (a) and III (b), with slight variations in DDT levels. The results are given in Table 7. Tuber infestation at the commencement of storage was lower than for the other two sections of this experiment, although this was somewhat compensated by a longer period of storage.

Table 6.

Experiment III (b): Arresting Infestation.

Treatment.	Percentage Tubers Infested.		
	Commencement of Storage.	End of Storage.	Increase.
1. DDT-treated sack (2·5% emulsion)	35·2	39·2	4·0
2. DDT-treated sack (1·0% emulsion)	24·0	42·9	19·0
3. DDT-treated sack (0·5% emulsion)	27·1	42·1	15·0
4. Control	41·2	94·4	53·1
Differences necessary for significance 5%	6·9	7·5	9·5
Differences necessary for significance 1%	9·6	10·3	13·2

Table 7.

EXPERIMENT III (c): ARRESTING INFESTATION.

Treatment.	Percentage Tubers Infested.		
	Commencement of Storage.	Secondary Inspection.	End of Storage.
	Dec. 29, 1950.	Feb. 16, 1951.	Mar. 12, 1951.
1. DDT 2·0% dust	12·2	8·0	10·9
2. DDT 1·5% dust	8·6	7·6	9·8
3. DDT 1·0% dust	13·6	18·3	11·7
4. DDT-treated sack (3·0% emulsion)	17·8	13·3	15·0
5. DDT-treated sack (2·0% emulsion)	10·6	9·0	11·1
6. DDT-treated sack (1·0% emulsion)	9·3	12·6	16·9
7. Control	13·3	29·1	45·7
Significant differences		1 to 6 << 7 1, 2 < 3	1 to 6 << 7

(<< = significantly less than, at 1% level; < = significantly less than, at 5% level).

Tuber breakdown was of little consequence as all decomposing tubers were removed before the counts on Feb. 16 and Mar. 13. This had the effect of reducing the infestation recorded for certain treatments on these dates.

All DDT levels, irrespective of method of application, arrested appreciable infestation in the tubers (Tables 5, 6 and 7). The highest level of DDT, whether as dust or impregnated in the container, was more efficacious when more than one in five tubers was infested at the commencement of storage (Tables 5 and 6).

(d) Experiment IV—Commercial Trial.

Results from Experiments I, II and III suggested 2 per cent. DDT dust or sacks impregnated with 2 per cent. DDT emulsion as efficacious levels for commercial use. Accordingly, a commercial trial was undertaken.

After applying treatments in the field, the tubers were placed in a bulk potato store and handled in accordance with normal commercial practices.

Table 8.

EXPERIMENT IV: COMMERCIAL STORAGE.

Treatment.	Percentage Tubers Infested.		
	Commencement of Storage.	Storage for 9 Weeks.	Storage for 15 Weeks.
1. DDT 2·0% dust	10·6	21·8	9·6
2. DDT-treated sack (2·0% emulsion)	12·5	24·8	11·8
3. Control	12·0	85·6	83·5
Significant differences		1, 2 << 3	1, 2 << 3

(<< = significantly less than, at 1% level).

The methods of assessing the results given in Table 8 are comparable with those that were obtained under experimental conditions. When examining for infestation after storage for nine weeks, all unsound tubers were discarded. Despite this, further infestation had developed when the experiment terminated, as under the system of sorting practised, some infested tubers were overlooked and returned to the bags. The influence of the DDT treatments, however, was obvious for the duration of storage: in the controls, few tubers were marketable at the end of the experiment.

(e) Experiment V—Alternative Methods of Applying DDT.

The impregnation of Chapman sacks with DDT and direct application of DDT dust to the tubers have several disadvantages, including lack of uniformity of application under farm conditions and residues on the tubers. Accordingly, an experiment including alternative methods of DDT application was designed. Details of treatments and results are given in Table 9.

Table 9.

Experiment V: Alternative Methods of Applying DDT.

Treatment.	Percentage Tubers Infested.		
	Commencement of Storage.	Storage for 6 Weeks.	Storage for 9 Weeks.
1. DDT 2·0% dust	2·0	1·6	5·5
2. DDT-treated sack (2·0% emulsion)	2·9	9·8	11·2
3. DDT-treated burlap strips	1·3	7·7	7·0
4. DDT-treated paper streamers	1·7	3·3	3·3
5. Control	0·2	4·2	5·2
Significant differences	5 << 2 5 < 1	1, 4 << 2; 1 << 2 5 < 2	4 << 2 5 < 2

(<< = significantly less than, at 1% level; < = significanty less than, at 5% level).

Unfortunately, initial infestations were low. Breakdown due to Irish blight (*Phytophthora infestans* de Bary) was considerable in this experiment and necessitated severe culling during the secondary inspection. This prevented the expected increase in tuber moth incidence. Breakdown was greatest in the dusted tubers and least in sacks containing burlap strips: by the end of storage, the former treatment was in poor condition. Further work will be necessary to evaluate tuber protection by these alternative methods of application.

(f) Experiment VI—Alternative Storage Containers.

The shortage of Chapman sacks in some years, and certain other undesirable features associated with this type of container, suggested the need for alternative containers. Only two types are readily available in the trade—standard hessian sugar-bags and heavy-duty, multiple-layer paper bags.

Table 10.

EXPERIMENT VI: ALTERNATIVE STORAGE CONTAINERS.

Treatment.	Percentage Tubers Infested.		
	Commencement of Storage.	End of Storage.	Increase.
Chapman sack—			
1. DDT 2·0% dust on tubers	17·1	26·2	9·1
2. DDT-impregnated (2·0% emulsion)	15·9	19·4	3·5
Sugar-bag—			
3. DDT 2·0% dust on tubers	7·9	15·9	8·0
4. DDT-impregnated (2·0% emulsion)	4·8	6·9	2·1
Paper bag—			
5. DDT 2·0% dust on tubers	21·8	25·8	4·0
6. DDT-treated (2·0% emulsion)	13·4	25·8	12·4
7. Control	27·0	69·1	42·1
Significant differences	4 << 1, 5, 7 3 << 5, 7 4 < 2, 6 2 < 7	1 to 6 << 7 4 << 1, 5, 6 4 < 2	

(<< = significantly less than, at 1% level; < = significantly less than, at 5% level).

Portions of the Chapman sacks were cut off so that holding capacity of all containers was similar. To ensure adequate ventilation in the paper bags, four evenly spaced holes of 1 in. diameter were punched in each side.

Results are set out in Table 10 in a form enabling comparisons of treatments within each container type, and in Table 11 to permit an evaluation of the containers and the application methods.

Table 11.

EXPERIMENT VI: ALTERNATIVE STORAGE CONTAINERS.

Treatment.	Mean Tuber Infestation.		
	Commencement of Storage.	End of Storage.	Increase
1. Chapman sack	16·5	22·7	6·2
2. Sugar-bag	6·3	11·0	4·7
3. Paper bag	17·4	25·8	8·4
DDT 2·0% dust	15·1	22·4	7·3
DDT-treated (2·0% emulsion)	10·8	16·5	5·7
Significant differences	2 << 1, 3	2 << 3 2 < 1	

(<< = significantly less than, at 1% level; < = significantly less than, at 5% level).

Despite a more open weave, the results obtained with sugar-bags were comparable with those from Chapman sacks. Paper bags were equally efficacious when the DDT was applied as a dust to tubers but were less so when it was applied to the container. Apparently, moths entered quite readily through the holes punched for ventilation and did not contact the DDT deposit before infesting tubers.

(6) Discussion.

At the strengths used, DDT, derris and pyrethrum-piperonyl butoxide as dusts and DDT-impregnated Chapman sacks and sugar-bags were all efficacious in protecting tubers when the initial infestations were less than 4 per cent. (Tables 3 and 4). Kaolin failed to arrest a comparable infestation.

All DDT treatments also satisfactorily arrested initial infestations from 8 to 35 per cent. (Tables 5, 6, 7, 8 and 11), although the higher levels of DDT were the more efficacious (Tables 5 and 6) when the initial infestation exceeded 20 per cent. These conclusions were biassed by the culling of damaged and rotting tubers at secondary inspections (Table 7).

Treating tubers with DDT dust at harvest is both practicable and effective under commercial conditions. The use of brown-pigmented dust largely overcomes the disadvantages of an unsightly white residue (Fig. 3). Breakdown, however, was more prevalent among dusted tubers, for in the presence of high humidity tubers retained moisture on their surfaces and this favoured the rapid spread of rot organisms.

The use of DDT-impregnated containers, though more expensive than dusting, is as effective as dusting and eliminates the unsightly dust residue. Tubers stored by this method remained drier and always opened in a more attractive condition than dusted tubers. In these experiments, however, shrinkage was evident following immersion of the containers in DDT emulsion. Passage through the mangle compressed the weave; this interfered with ventilation and, in turn, promoted the accumulation of moisture whenever rots developed.

Although Chapman sacks and sugar-bags proved equally suitable as storage containers, the latter, because of the more open weave, were seldom associated with breakdown. Paper bags, on the other hand, were impractical for lengthy storage. Moisture from decomposing tubers wet the paper and in extreme circumstances caused bag collapse.

Infestation from outside the containers was shown (Expt. II) to be of little significance. In this case, containers were tightly sewn after the tubers were added. The commercial practice of filling a bag so that the mouth is not closed entirely provides an easy means of moth entry, irrespective of the type of weave in the container.

The likelihood of appreciable levels of infestation in commercial potatoes at harvest in years that favour *Gnorimoschema* activity would require levels of DDT for field application commensurate with the more effective treatments in these experiments. Both 2 per cent. dust and containers impregnated with 2 per cent. emulsion proved adequate under commercial conditions (Expt. IV).

The storing of good-quality sound tubers under well-ventilated conditions, coupled with the periodic sorting out of decomposing tubers, should eliminate the drawbacks to either method of DDT application mentioned above.

III. DDT RESIDUES.

Experiments to determine DDT residues on containers and on tubers were carried out concurrently with those dealing with tuber protection.

Methods of sampling and the reasons for using DDT in emulsion form for impregnating containers are discussed in Appendix II.

(1) Methods and Assessing Results.

(a) Residues in Containers.

The method of treating containers and sampling for residues, devised from preliminary investigations (Appendix II), was standardised and was followed throughout these studies.

Residue determinations were made at the commencement and end of Experiments III (c), V and VI. The burlap strips used in Experiment V were sampled at the end of storage; the four unused strips provided the samples at the commencement of the experiment.

When preparing containers for Experiments III (c) and VI, four extra sampling sacks or bags were treated at each level of DDT emulsion for residue determinations at the commencement and the end of storage. Two additional sacks were impregnated with 2 per cent. DDT emulsion; the six sampling containers provided residue values for this treatment in Experiments III (c) and V.

At the end of storage, samples taken from the sampling containers as well as from the containers used to store the tubers provided a means of appreciating loss of residue due to handling. During storage, the sampling containers were left undisturbed in the storage shed.

(b) Residues on Tubers.

DDT residues on tubers at the end of storage were determined at the conclusion of Experiment I. Thirty tubers from the 2 per cent. DDT dust treatment and 40 from DDT-impregnated sacks provided samples for the analyses. Thirty tubers from the control sacks were included as blanks in the DDT determinations.

The persistence of the DDT deposit on tubers following various combinations of peeling and washing prior to cooking was investigated. A suitable quantity of commercially dusted tubers was obtained from storage. Samples of seven potatoes each with five replications were subjected to six different treatments of peeling and/or washing. All washing was carried out in still water, about two pints being used for each 1 lb. sample of tubers. Undusted tubers were similarly treated for comparison. Following treatments, the tubers were analysed for DDT residues. The various treatments are detailed in Table 12.

Table 12.

DDT Residues at Commencement and End of Storage.
Experiments III (c) and V (Chapman Sacks)—(g./sq. ft.).

Replicates.	1% DDT Emulsion.			2% DDT Emulsion.					3% DDT Emulsion.		
	Sample Bags.		Expt. III (c) End.	Sample Bags.		Expt. III (c) End.	Expt. V End.	Expt. V (Burlap) End.	Sample Bags.		Expt. III (c) End.
	Commencement.	End.		Commencement.	End.				Commencement.	End.	
A	1·06	·98	·40	1·20	·77	1·16	·63	·98	2·16	1·57	1·34
B	1·29	·39	·28	·92	·99	1·00	·94	1·25	1·96	1·84	1·70
C	·88	·39	·68	·97	·98	·93	·89	·99	1·83	1·66	1·68
D	1·06	·67	·49	·80	·94	·85	1·08	1·25	1·98	1·92	1·96
E	..	..	..	1·23	·99	..	..	..	..	..	..
F	..	..	..	·69	·99	..	..	..	..	..	..
Average ..	1·07 (1)	·61 (2)	·46 (3)	·97	·94	·98	·88	1·12	1·98 (1)	1·75 (2)	1·67 (3)
	1 >> 2, 3			No significant differences					1 > 3		

Experiment VI (2% DDT Emulsion).

Replicates.	Chapman Sacks (g./sq. ft.)			Sugar-bags (g./sq. ft.)			Paper Bags (mg./sq. ft.)		
	Sample Bags.		Expt. Bags End.	Sample Bags.		Expt. Bags End.	Sample Bags.		Expt. Bags End.
	Commencement.	End.		Commencement.	End.		Commencement.	End.	
A	·96	1·17	1·21	·86	·38	·41	134	128	83
B	1·02	1·21	·94	·45	·23	·72	137	89	108
C	*	1·52	1·03	·44	·39	·52	135	89	111
D	*	1·08	·98	·28	·49	·49	136	111	89
Average	·99	1·24	1·04	·51	·37	·54	136 (1)	104 (2)	98 (3)
	No significant differences			No significant differences			1 >> 2, 3		

(* = samples lost; >> = significantly greater than, at 1% level; > = significantly greater than, at 5% level.)

(2) Results.

(a) Residues on Containers.

The residue analyses of the sample discs from the extra sampling containers and those used in Experiments III (c), V and VI are set out in Table 12. The results are expressed as either grams or milligrams of *p.p.'* isomer per sq. ft.

Values of ·17, ·023 and nil were found in blank tests for Chapman sacks, sugar-bags, and paper bags respectively. No allowance has been made for these results in the values set out in Table 12.

Although sample bags remained undisturbed during storage periods, a significant reduction in DDT deposit had occurred at the final sampling for those treated with 1 per cent. DDT emulsion and for the paper bags used in Experiment VI.

A loss of residue also occurred in experimental bags treated with the lowest and highest DDT concentration in Experiment III (c) and for Chapman sacks and paper bags in Experiment VI. Among replicates, however, variability of residue was quite pronounced at the initial and final sampling, irrespective of container type and level of DDT concentration used.

Although differences in values between commencement and end of storage may be partly explained by deficiencies in the technique of sampling, it can be assumed that a portion of the DDT deposit in containers is dislodged during their handling.

(b) Residues on Tubers.

The mean values for residues on tubers from Experiment I at the end of storage are given in Table 13, and representative tubers are shown in Fig. 3.

Table 13.

DDT Residues on Tubers after 13 Weeks' Storage.

Treatment.	No. of Tubers Sampled.	Mean DDT Concentration (mg./100 g.).
DDT-treated sack (2·0% emulsion)	40	8·8
DDT 2·0% dust	30	9·2
Control	30	1·0

These tubers had been sampled for tuber moth infestation on three occasions prior to the taking of the samples for DDT determinations. This handling had resulted in a considerable transfer of DDT from the treated sack to the tubers, so much so that the analysis revealed a value comparable with that for the dusted tubers.

The results of the DDT determinations on tubers receiving various treatments of peeling and/or washing are given in Table 14.

Table 14.

DDT Residue (p.p.m.) on Potatoes Prepared for Cooking.

Treatment.	Replicates.				
	A.	B.	C.	D.	E.
1. Peeled only	3·8	8·9	3·6	2·1	0·9
2. Washed, peeled and washed again in same water	2·9	2·0	3·3	1·8	0·9
3. As for 2, except washed again in clean water	Nil	1·5	1·5	Nil	0·9
4. Not peeled or washed	2·5	4·1	3·2	6·0	4·4
5. Washed but not peeled	0·8	Nil	0·8	Nil	0·8
6. Peeled, then washed in clean water ..	Nil	4·7	0·9	Nil	2·9
7. Undusted potatoes	Nil	Nil	Nil	Nil	Nil

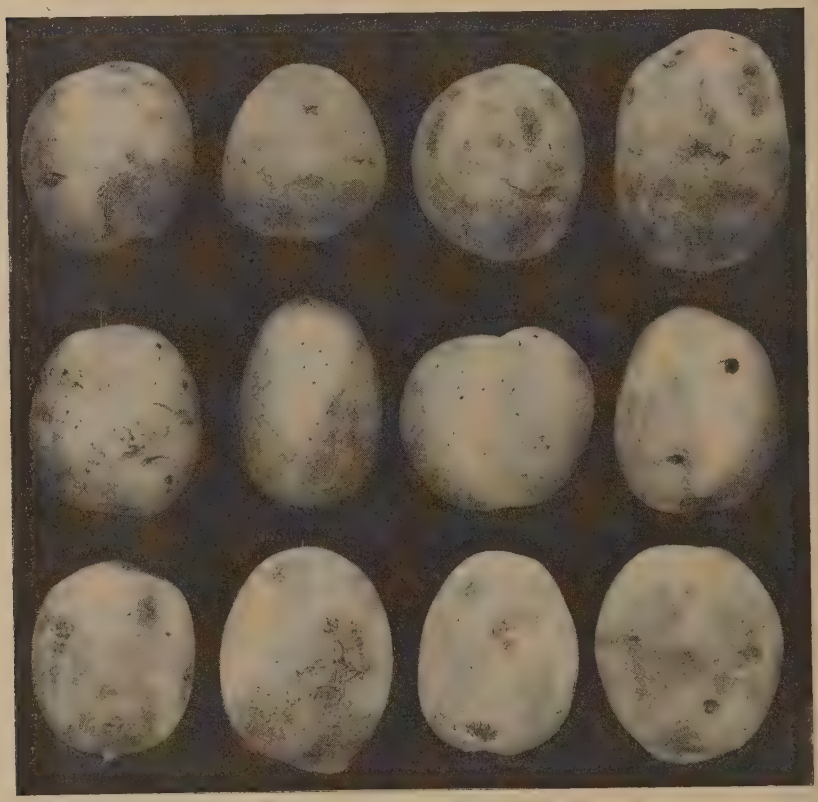

Fig. 3.

Residues on Tubers. From top downwards:—DDT brown-pigmented dust; washed; DDT non-pigmented dust.

Under the conditions of the experiment, washing alone reduced the residue to within the limits of DDT tolerance. When during peeling the cut surface of the tuber is contaminated with DDT, the deposit is more difficult to remove. Washing in clean water, whether before or after peeling, reduces the deposit to comparable levels.

(3) Discussion.

The mean DDT residues per square foot retained by both the Chapman sacks and sugar-bags (Appendix II) are roughly proportional to the emulsion concentrates used, though the more heavily woven Chapman sack retained a greater quantity of DDT per square foot than the sugar-bag. Hofmaster (1949) found that burlap dipped in a 5 per cent. DDT-xylene solution retained an average DDT deposit of 1·29 g. per sq. ft., a figure comparable with that obtained for sugar-bags treated with the same DDT concentration in these experiments.

Also, mean values for DDT residues per square foot for both Chapman sacks and sugar-bags treated with DDT emulsion do show some relationship in both the preliminary studies (Appendix II) and subsequent experiments (Table 12). Corresponding values for paper bags, however, are not comparable.

Despite these broad relationships between residues due to containers on the one hand and DDT concentrates on the other, many inconsistencies were obvious from the results (Table 12). Though the methods of treating containers and sampling for DDT residues were standardised throughout, scope for variation existed in both these processes.

The varying thickness of seam and variation in weave, by influencing the evenness of DDT deposit retained, would account for some variation among values for DDT deposit for related containers, more particularly for Chapman sacks. Also, unevenness of weave may have contributed to discrepancies between samples for chemical analysis. More consistent values were obtained for the more uniformly woven and thinner sugar-bags.

Though Chapman sacks and sugar-bags exhibited variations in residue among replicates within treatments, and also retained dissimilar quantities of DDT due to weight and closeness of weave, both containers proved suitable for DDT impregnation. Paper bags, apart from unsuitability as containers under moist conditions, did not lend themselves to uniform treatment with DDT emulsion, lost the DDT deposit more readily than the jute containers and could not withstand handling. This type of container can be ruled out of consideration for storing potato tubers.

Butterfield, Parkin and Gale (1949) found that DDT was transferred from the container to the foodstuff if the sacking contained a relatively high concentration of DDT or the sack was roughly handled, although the effect of handling was variable. In these studies, despite possible variation due to sampling and chemical methods adopted, loss of residue followed handling of containers (Table 12), but reductions were not consistent with degree of handling.

Appreciable quantities of the DDT lost from containers during handling were transferred to the tubers (Table 13). Hofmaster (1949) showed that potatoes in contact with burlap treated with 3 per cent. DDT retained an average DDT deposit of 3·05 p.p.m. Atkins and Greer (1953), investigating the storage of corn in jute bags treated with DDT, found 13·8 p.p.m. transferred from fabric impregnated with 1 per cent. DDT and 23·0 p.p.m. associated with 1·5 per cent. DDT. Thus storing tubers in treated containers, though overcoming the obvious deposit associated with DDT dust, does not reduce the hazard of DDT residue.

The ultimate fate of residues on tubers is of considerable importance to the consumer. It would be impossible to obtain a consistent figure for the DDT deposit persisting after tubers had undergone the handling associated with transport and sale to the consumer. Those used for the peeling and washing tests were taken direct from storage and did not receive the extra handling received by trade potatoes. Thus the residues would exceed greatly those obtaining in normal practice.

The DDT residue persisting after storage is superficial. Butterfield, Parkin and Gale (1949) had shown that fatty foodstuffs stored in DDT-treated containers absorbed DDT through the unbroken skin. In these experiments washing alone removed the greater proportion of the DDT and DDT recovered from peeled tubers had been transferred during peeling operations.

It is concluded that following normal washing and peeling prior to cooking, tubers lose most of the DDT still persisting after storage and handling.

IV. GENERAL CONCLUSIONS.

Effective field control of *Gnorimoschema,* rapid harvesting, the prompt culling of unsound tubers and the application of measures designed to prevent tuber moth activity in bagged tubers are essential to the successful storage of potatoes in Queensland. Under the system of grading, culling and bagging practised in the field, the inclusion of infested tubers can scarcely be avoided. This nucleus of infestation may be appreciable in seasons of major pest activity. The prompt application of measures that will arrest and destroy this initial infestation and prevent reinfestation is of prime importance whether tubers are stored as seed or for table use.

In these experiments, a 2 per cent. DDT dust applied directly on the tubers at the rate of ½ lb. per bag, and placing tubers in either Chapman sacks or sugar-bags previously treated in a 2 per cent. DDT emulsion, were the most practical and efficacious means of protecting tubers. Both methods, also, proved entirely satisfactory under commercial conditions (Experiment IV) and were recommended for general use (May 1951). Their benefit is enhanced, however, if unsound tubers are graded out before bagging and tubers are stored under cool, well-ventilated conditions.

The use of inert dusts as protectants, or of non-toxic insecticides such as derris and pyrethum-piperonyl butoxide, cannot be advocated in preference to DDT for *Gnorimoschema* control. Their dependence on low humidity, low

initial infestation, and the unlikelihood of reinfestation during storage for the successful protection of tubers detracts from their general adoption. Further, their use would not compensate for any of the bad features associated with the use of DDT.

When considered from the viewpoint of cost, ease of application, keeping quality of tubers in storage and appearance, each method of DDT application has its advantages. DDT dust, however, is preferable for protecting seed potatoes, especially when they are held in open trays or bulked on the shed floor.

Tubers treated with DDT dust or stored in DDT-impregnated containers may carry an appreciable DDT deposit at the termination of storage. The system of handling tubers from storage to the consumer, however, together with the several processes involved in their preparation for the table, reduces the DDT residue below the limit of tolerance for health standards.

V. ACKNOWLEDGEMENTS.

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APPENDIX I.

Sampling Tubers.

In the following analyses the transformed variate, ϕ, defined by the relation

$$p=100 \sin^2 \phi,$$

where p is the percentage, has been used. The results are based on data from the 1949-50 series of experiments, viz. II, III(a) and III(b), excluding the control in the last experiment for the determination of inter-bag variability.

Mean Squares.

Expt.	D.F.	Whole Plot Error.	D.F.	Sampling Error.
II ..	12	113·90	175	107·01
III (a) ..	20	120·81	210	108·58
III (b) ..	15	116·84	168	102·01
Average ..	47	117·78	553	106·09

It is assumed that the variability is made up of two parts, viz., an intra-bag component (variance σ_1^2) and an inter-bag component (variance σ_2^2). The sampling error mean square provides an estimate of σ_1^2. The whole plot error mean square provides an estimate of $(1-f)\ \sigma_1^2 + 8\ \sigma_2^2$, where f is the fraction of each bag sampled. The method adopted corresponds to a value of $f=\cdot 22$ approximately, and this value gives 4·38 as an estimate of σ^2.

These estimates can be used to assess the accuracy of various methods of sampling. If 10n potatoes per bag are sampled and there are r replicates of each treatment, the estimated necessary difference for significance at the 5% level will be

$$\sqrt[3]{\left[\frac{1}{r}\left\{\frac{(1-f)}{n}\sigma_1^2+\sigma_2^2\right\}\right]},$$

where f is the fraction sampled and depends on n. The values of this quantity for various values of r and 10n are tabulated below.

Replications.	Number Counted per Bag (10n).							
	20.	40.	60.	80.	100.	160.	240.	All.
4	11·1	7·9	6·6	5·8	5·2	4·3	3·6	3·1
5	9·9	7·1	5·9	5·2	4·7	3·8	3·2	2·8
6	9·0	6·5	5·4	4·7	4·3	3·5	3·0	2·6
8	7·8	5·6	4·6	4·1	3·7	3·0	2·6	2·2
10	7·0	5·0	4·1	3·6	3·3	2·7	2·3	2·0
12	6·4	4·6	3·8	3·3	3·0	2·5	2·1	1·8

Further discussion of sampling methods involves other than statistical considerations, e.g. the relative cost of different methods.

The above estimates are given in terms of the transformed variate. The equivalent percentages depend on the average percentage and are greatest in the neighbourhood of 50 per cent. The equivalent percentages corresponding to unit intervals of ϕ at 50 per cent. and 25 per cent. are given below.

Average Percentage.	Necessary Difference (ϕ).									
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
50 ..	1·8	3·5	5·2	7·0	8·7	10·4	12·1	13·8	15·5	17·1
25 ..	1·5	3·1	4·6	6·1	7·5	9·1	10·5	12·0	13·4	14·8

From the above a method of sampling was chosen commensurate with practicability. It was found more convenient to handle 4–6 replicates of each treatment and the sampling was regulated accordingly.

APPENDIX II.

Sampling Containers for Residue Analyses.

Preliminary studies were undertaken to investigate methods of treating and sampling containers for residue determinations thereby enabling an appreciation of bag-to-bag variation of residues. This work also provided a comparison between emulsion and dispersible powder DDT for treating containers.

Two series of containers, each comprising four Chapman sacks, sugar-bags and paper bags, were treated, one with emulsion and the other with dispersible powder. Concentrations used are given in Table 15.

When dry, five paired 1 in. diameter discs were punched from each container. The pattern of sampling was standardised throughout. To obtain the sample discs, the punch was given several sharp taps with a hammer, after which those strands not severed were cut with a knife. For paper bags, only the centre pair of discs from each punching was retained for analysis.

In addition, four untreated of each of the three types of containers provided blanks in the DDT determinations.

The results of DDT analyses, expressed as *p.p'* isomer DDT per sq. ft., are given in Table 15. Corrections have been made for the chlorine content (0·02 g./sq. ft.) of both Chapman sacks and sugar-bags.

Table 15.

DDT Residue Determinations.

Container.	Chapman Sacks. (g./sq. ft.).			Sugar-bags. (g./sq. ft.).			Paper Bags. (mg./sq. ft.).		
	DDT Emulsion.								
	2%	3%	5%	2%	3%	5%	2%	3%	5%
A	1·20	1·42	3·30	·70	·76	1·28	294	362	341
B	1·29	1·51	3·56	·68	·76	1·46	242	343	340
C	1·16	1·72	3·78	·55	·84	1·46	225	344	287
D	1·51	1·78	3·25	·55	·81	1·16	242	248	338
Means	1·29	1·61	3·49	·64	·79	1·34	251	324	326
	DDT Dispersible Powder.								
	2%	3%	5%	2%	3%	5%	2%	3%	5%
A	·92	2·42	1·82	·83	1·03	*	309	*	158
B	·93	2·54	1·53	·93	·93	·96	261	249	247
C	1·24	1·72	2·77	·58	1·07	1·47	261	257	308
D	·62	1·82	2·02	·78	1·18	1·23	158	155	154
Means	·93	2·13	2·04	·78	1·05	1·22	247	224	219

* = Samples lost.

Emulsions were retained by containers as fairly uniform dressings with no obvious residues. Unsightly white residues were obvious on containers treated with dispersible powder; the non-permanence of these deposits, particularly on paper bags, was evident during sampling and is reflected in the variations amongst replicates.

As a result of this preliminary work, emulsions only were used in all experiments and variance and fiducial limits for residues on containers are given in Table 16.

Table 16.

VARIANCE AND FIDUCIAL LIMITS FOR RESIDUES ON CONTAINERS.

Container.	D.F.	Variance per Container.	95% Range for Mean Based on		
			1 container.	4 containers.	9 containers.
Sugar-bags ..	9	·0102	± ·23 g.	± ·11 g.	± ·08 g.
Chapman Sacks ..	9	·0347	± ·42 g.	± ·21 g.	± ·14 g.
Average	18	·0224	± ·32 g.	± ·16 g.	± ·11 g.
Paper Bags ..	9	·1417	± ·85 mg.	± ·43 mg.	± ·28 mg.

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STUDIES ON CODLING MOTH CONTROL IN THE GRANITE BELT.

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SUMMARY.

The literature on Cydia pomonella **(L.) in Queensland is reviewed.**

The details of six orchard trials during 1949-50, 1954-55 and 1956-57 are given. The materials used in the several spray programmes were DDT, DDD (TDE), diazinon, dieldrin, endrin, malathion, methoxychlor, and parathion. Treatments were applied according to pre-determined programmes for each season, additional sprays, either alone or in combination, being used to control pests other than codling moth.

The results illustrate the variation in codling moth populations between orchards and seasons. Under the conditions of these trials none of the newer materials tested gave better results than DDT. It was concluded, therefore, that alterations to the existing codling moth spray programme cannot be justified.

I. INTRODUCTION.

Codling moth, *Cydia pomonella* (L.), was first recorded in Queensland infesting fruit in Brisbane (Tryon 1889). Most investigational work, however, with this serious pest has been carried out in districts centred on Stanthorpe in the Granite Belt, the commercial apple-producing area of the State.

Jarvis (1933, 1935, 1937) reported experiments and observations on control with insecticides, bandaging and lure trapping. Arsenate of lead, and a combination of white oil and nicotine sulphate, were among the most effective chemical controls. Ward and Ross (1938) further tested these and other combinations of spray materials, and also made observations on the seasonal activity of moths, using lure traps to sample populations with a view to devising a practicable method of timing spray applications. Ward and Groom (1940) conducted a spray trial comparing the efficacies of a fixed 3-weekly application programme with a programme in which the timing of spray applications was based on lure trap data. These authors concluded that lure trapping could form the basis for district advisory notices intimating the most suitable times to apply sprays. Caldwell (1948), following this system of spray timing, demonstrated that the use of DDT 0·1 per cent. was a distinct improvement on previous recommendations.

May (1948) confirmed the superiority of DDT, and suggested that reduction in cover sprays from six to four would not prejudice control. Alternative programmes covering a range of DDT concentrations were then tested (May 1949). It was also demonstrated (May 1950) that the calyx spray was not required when spring moth populations were small.

From these earlier trials and those for the 1949-50 trial reported in this paper, May (1952) concluded that the application of two sprays of DDT 0·1 per cent., three weeks apart, made to coincide with each of the two periods of major moth activity, was commercially desirable.

Commercial spray programmes covering the control of all major pests of apples were issued by May (1952) and May and Bengtson (1955). Although these satisfied the immediate needs of orchardists, disadvantages associated with the use of DDT in apple orchards and the advent of newer chemicals warranted continued studies; results of relevant orchard trials are reported in this paper.

II. MATERIALS AND METHODS.

(1) Materials.

The materials used were:—

DDT.—An emulsion concentrate containing 25 per cent. w/v *p.p'* isomer.

Diazinon.—An emulsifiable preparation containing 20 per cent. w/v active ingredient.

Dieldrin.—An emulsifiable preparation containing 16 per cent. w/v active ingredient.

Endrin.—An emulsifiable preparation containing 20 per cent. w/v active ingredient.

HETP.—An emulsifiable preparation containing 16–20 per cent. w/v *TEPP* as active ingredient.

Lead arsenate.—A powder containing 31 per cent. As_2O_5 as lead arsenate, and 1 per cent. dispersing agent; containing less than 0·5 per cent. water-soluble arsenic compounds.

Malathion.—An emulsifiable preparation containing 50 per cent. w/v active ingredient.

Methoxychlor.—An emulsifiable preparation containing 24 per cent. w/v active ingredient.

Nicotine sulphate.—A concentrate containing 40 per cent. w/w nicotine as nicotine sulphate.

Parathion.—An emulsifiable preparation containing 50 per cent. w/v active ingredient.

DDD (TDE).—An emulsion concentrate containing 30 per cent. w/v *p.p'* isomer.

Thiram.—A dispersible powder containing 80 per cent. w/w active ingredient.

Urea.—Containing 46 per cent. nitrogen as urea.

White oil.—Containing refined mineral oil, 74 per cent. w/v (82·5 per cent. by weight: unsulphonatable residue not less than 90 per cent.).

(2) Design of Trials.

Randomised blocks with a plot size of either one or two trees were used.

(3) Treatment Application.

Treatments were applied at a pressure of 200–250 lb. per sq. in., using a small power spray with a hand-operated lance. Complete tree cover was aimed at.

(4) Assessing Results.

Windfall and harvested fruit from plot trees were examined for larval damage. Where large numbers of harvested fruit were available random samples were taken.

A fruit was recorded as sound if there was no evidence of codling moth damage. Once damage was evident the fruit was classified as stung. In most trials the stung fruit were subdivided into blind stings, if larvae failed to develop, and wormy, if larvae developed to maturity.

Layout, spray combinations, detailed programmes, treatment application dates and other relevant data are given with the results.

III. RESULTS.

(1) 1949-50 Trials.

A 9 x 5 randomised block with single-tree plots of the variety Granny Smith was established on each of two orchards.

Sprays used were DDT 0·1 per cent.; lead arsenate 3 lb./100 gal.; HETP 1:800 with spreader; and parathion 0·02 per cent. (except where indicated as 0·04 per cent.).

Table 1.

SPRAY PROGRAMMES FOR 1949–50 TRIALS.

Programme.	Cover Sprays.					
	1 Nov. 8.	2 Nov. 30.	3 Dec. 27.	4 Jan. 10.	5 Jan. 31.	6 Feb. 2.
A	DDT	DDT	DDT + HETP	DDT	DDT	DDT
B	DDT	DDT + lead arsenate	HETP	DDT	DDT + lead arsenate	..
C	DDT	DDT	HETP	DDT	DDT	..
D	DDT	Parathion (0·04%)	..	DDT	DDT + lead arsenate	..
E	DDT	DDT + parathion	..	DDT	DDT + lead arsenate	..
F	DDT	DDT + HETP	..	DDT	DDT + lead arsenate	..
G	Parathion	Parathion	..	Parathion	Parathion	..
H	Parathion (0·04%)	Parathion	..	Parathion (0·04%)	Parathion	..
I	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated

Prior to treatments all plots in both trials received uniform treatment as follows:—Trial 1: a semi-dormant spray (pale oil 4 gal., lime sulphur 4 gal., water 80 gal.); Trial 2: semi-dormant oil (1 in 16), and a calyx spray (lead arsenate paste 6 lb., lime sulphur 1½ gal., hydrated lime 6 lb., water 100 gal.).

Programmes with the standard (Programme A) consisting of six DDT applications are given in Table 1.

All windfall fruit were examined. Of the harvested fruit, on Trial 1 two loose bushels and on Trial 2 three loose bushels were taken as a sample per plot. Trial 1 was harvested on Apr. 18 and 19 and Trial 2 on Mar. 29 and 30: results are given in Table 2.

Table 2.

Results of 1949–50 Trials.

Programme.	Trial 1.				Trial 2.			
	Harvested Crop.		Total Crop.		Harvested Crop.		Total Crop.	
	Wormy.	Stung.	Wormy.	Stung.	Wormy.	Stung.	Wormy.	Stung.
	%	%	%	%	%	%	%	%
A	0·0	0·0	0·1	0·1	0·7	2·7	1·1	3·1
B	0·4	0·8	0·4	0·9	0·5	3·2	1·2	3·7
C	0·7	1·6	1·0	2·0	0·4	2·6	0·7	2·8
D	0·7	1·1	1·2	1·6	1·7	4·9	3·3	6·2
E	0·7	1·0	0·9	1·1	1·0	3·5	1·7	4·1
F	0·5	1·5	0·8	1·9	1·5	7·4	2·6	8·3
G	4·0	4·7	4·5	5·4	3·7	6·4	3·9	6·5
H	1·9	3·1	2·1	3·4	2·3	4·4	2·7	4·6
I	4·0	4·4	5·7	6·1	10·8	15·1	16·3	20·1
Differences necessary for significance 5%	3·0	3·2	3·3	3·4	3·4	5·8	3·7	5·9
Differences necessary for significance 1%	4·1	4·3	4·4	4·5	4·6	8·8	4·9	7·9

Codling moth infestations in these trials were not heavy and all treatments were significantly better than untreated.

(2) 1954-55 Trials.

An 8 x 4 randomised layout was used with single-tree (Delicious) plots in Trial 1, 2-tree (Jonathan) plots in Trial 2, and two separate blocks with single-tree (Granny Smith) plots as Trial 3.

Sprays used were DDT 0·1 per cent.; endrin 0·025 per cent. and 0·05 per cent.; dieldrin 0·05 per cent.; lead arsenate 3 lb./100 gal.; white oil 2½ pt./100 gal.; nicotine sulphate 1¼ pt./100 gal.; DDT 0·1 per cent.; thiram 1½ lb./100 gal.; urea 5 lb./100 gal.; and parathion 0·01 per cent.

Prior to treatments all plots in the trials received uniform applications of sprays. These included:—Trial 1, superior oil 3 in 100 while dormant; Trial 2, superior oil 3 in 100 while dormant, lime sulphur 1 in 30 at "pink", and lead

Table 3.

SPRAY PROGRAMMES FOR 1954–55 TRIALS.

Programme.	Cover Sprays.					
	1.	2.	3.	4.	5.	
	All Trials.	All Trials.	All Trials.	All Trials.	Trials 1 and 3.	Trial 2.
A	DDT	DDT + Lead arsenate + Parathion	Parathion	DDT	DDT + Lead arsenate + White oil	DDT + White oil + Nicotine sulphate
B	DDT	DDT + DDD + Parathion	Parathion	DDT	DDT + DDD	DDT + DDD
C	Endrin 0·025%	Endrin 0·025% + Lead arsenate + Parathion	Parathion	Endrin 0·025%	Endrin 0·025% + Lead arsenate	Endrin 0·025% + White oil + Nicotine sulphate
D	Endrin 0·025%	Endrin 0·025% + DDD + Parathion	Parathion	Endrin 0·025%	Endrin 0·025% + DDD	Endrin 0·025% + DDD
E	Endrin 0·05%	Endrin 0·05% + Parathion	Parathion	Endrin 0·05%	Endrin 0·05%	Endrin 0·05%
F	Dieldrin	Dieldrin + Parathion	Parathion	Dieldrin	Dieldrin	Dieldrin
Ga .. .. .. Trial 1 and Trial 3 (Block 1)	Programme A + Urea	Programme A + Urea	Parathion	Programme A + Urea	Programme A + Urea	..
Gb .. .. .. Trial 2 and Trial 3 (Block 2)	Programme A + Thiram	Programme A + Thiram	Parathion	Programme A + Thiram	Programme A + Thiram	..
H	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated

Table 4.

RESULTS OF 1954–55 TRIALS.

Programme.	Percentage Fruit Stung.							
	Trial 1.				Trial 3.			
					Block 1.		Block 2.	
	Jan. 17.		Entire Crop.		Entire Crop.		Entire Crop.	
	Transformed Mean.	Equivalent Mean.	Transformed Mean.	Equivalent Mean.	Transformed Mean.	Equivalent Mean.	Transformed Mean.	Equivalent Mean.
A	7·7	1·8	14·4	6·1	53·2	64·1	38·2	38·3
B	7·2	1·6	13·6	5·5	50·7	59·8	34·9	32·7
C	13·0	5·1	20·0	11·7	65·2	82·4	53·9	65·2
D	15·1	6·8	21·9	13·9	69·9	87·6	54·8	66·8
E	13·1	5·1	27·4	21·2	65·8	83·1	49·9	58·5
F	24·9	17·7	34·0	31·4	73·5	91·9	61·9	77·8
G	3·8	0·4	10·7	3·5	50·7	59·9	34·3	31·7
H	24·6	17·3	42·4	45·5	71·0	89·4	61·4	77·0
Differences necessary for significance 5%	7·5		9·9		8·8		6·7	
Differences necessary for significance 1%	10·2		13·5		11·9		9·2	

arsenate 3 lb., hydrated lime 3 lb., casein spreader 1 lb., colloidal sulphur 2 lb., lime sulphur ½ in 100 of water at "calyx"; Trial 3, superior oil 3 in 100 while dormant.

Programmes are given in Table 3, and application dates of cover sprays were as follows:—

Trial 1: Nov. 8, Nov. 26-27, Dec. 12, Jan. 15, and Feb. 3;

Trial 2: Nov. 2, Nov. 23, Dec. 6, Jan. 1, and Feb. 1;

Trial 3: Nov. 6, Nov. 24, Dec. 5–6, Jan. 1, and Feb. 2.

As heavy stinging was occurring, and at the request of the orchardist, an additional DDT treatment was applied on Jan. 20 to trees concerned with spray programmes C, D, and F in Trial 1.

Infestation was negligible in Trial 2. Trial 1 was harvested on Mar. 31 and Trial 3 during the period Apr. 20 to May 5. In Trial 1 a progress count of stung fruit was made on Jan. 17. All relevant results are given in Table 4.

The use of DDT gave better protection than the other materials, but none of the programmes was entirely satisfactory in controlling the heavy pest populations encountered. The addition of either urea or thiram did not interfere with the insecticidal action of DDT sprays.

(3) 1956-57 Trial.

This consisted of an 8 x 4 randomised block with single-tree plots of the variety Granny Smith.

Table 5.

Results of 1956–57 Trial.

Treatment.	Programme.	Percentage Fruit Stung.		Percentage Blind Stings/ Total Stings.	
		Transformed Mean.	Equivalent Mean.	Transformed Mean.	Equivalent Mean.
A. DDT 0·1%	Recommended	21·8	13·8	38·2	38·3
B. Parathion 0·01% ..	Recommended	34·6	32·2	28·2	22·3
C. Malathion 0·05% ..	Recommended	30·0	25·1	27·4	21·2
D. Diazinon 0·1% ..	Recommended	23·8	16·2	35·5	33·7
E. Methoxychlor 0·1% ..	Recommended	24·0	16·5	22·1	14·2
F. DDT 0·1%	Fortnightly	18·6	10·2	51·0	60·4
G. Malathion 0·05% ..	Fortnightly	24·6	17·3	34·5	32·1
H. Untreated	..	37·2	36·6	9·4	2·7
Differences necessary for significance 5%		8·9		12·7	
Differences necessary for significance 1%		12·1		17·3	

Sprays used are given with results in Table 5. Applications of treatments A to E were made in accordance with the recommended apple pest spraying programme (May and Bengtson 1955). Treatments F and G were applied fortnightly.

Prior to treatments all plots received uniform applications of superior oil 3 in 100 while dormant, and Bordeaux 10–10–100 at green tip.

During the trial all trees received a spray of DDD 0·1 per cent. on Nov. 27 and two sprays of parathion 0·01 per cent. on Nov. 27 and Dec. 11 to control incidental pests.

Treatments A to E were applied on Nov. 4 and 7 and Jan. 8 and 25; treatments F and G were applied on Nov. 4 and 22, Dec. 6 and Jan. 3 and 17. Harvesting was carried out between Apr. 30 and May 15, and results are given in Table 5.

With this moderate infestation none of the materials was better than DDT although diazinon was almost as good.

IV. GENERAL COMMENTS.

These trials illustrate the variations in codling moth infestation from orchard to orchard and from season to season in the Stanthorpe district and the difficulty of holding damage by heavy infestations to an economic level.

The levels of population existing in trials from untreated and inferior treatment plots provided ideal conditions for comparing the several treatments and programmes. None of the newer materials tested has given better results than DDT, but diazinon and methoxychlor may warrant additional work. The results indicate also the need for long-term detailed investigations into the timing of sprays. The effect of treatments on other pests of apples, especially fruit fly, *Strumeta tryoni* (Frogg.), and mites, *Tetranychus* spp., should not be overlooked in future codling moth control studies.

At present, alterations in the codling moth spray programme of May and Bengtson (1955) are not indicated.

V. ACKNOWLEDGEMENTS.

Orchardists provided the trial sites, and statistical analyses were carried out by Mr. P. B. McGovern, Senior Biometrician. This assistance is gratefully acknowledged.

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STRAWBERRY ROOT-KNOT NEMATODE INVESTIGATIONS IN QUEENSLAND.

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SUMMARY.

Studies on the root-knot nematode Meloidogyne hapla **Chitwood, a common pest of strawberries in south-eastern Queensland, are reported.**

The use of nematode-infested planting material results in later cropping, reduced plant size and lower yield. Mean berry size is not reduced.

Treatment of nematode-infested runners in hot water at 47 deg. C. for 6, 10 and 14 min. controls the strawberry root-knot nematode but delays fruiting and does not increase overall yields.

The use of dips containing chlorophenyl mercury choroglucine, phenyl mercury dinaphthylmethane disulphonate, phenyl mercury salicylate or mercuric chloride on nematode-infested runners prior to planting controls nematodes but subsequent plant growth is unsatisfactory.

Vigorous runners relatively free of nematodes can be produced in infested fields by allowing rooting to proceed in a 6 in. mulch of sawdust spread between the parent rows.

I. INTRODUCTION.

Commercial strawberry growing in Queensland is restricted to the south-eastern portion of the State. Runners are planted in March and harvesting extends from June to December, when the plants are removed or thinned for the production of runners.

The strawberry root-knot nematode, *Meloidogyne hapla* Chitwood, is to be found in most plantings (Colbran 1958). Two types of galls occur on the roots, viz., small galls with lateral rootlets on the fibrous roots and larger terminal galls on the main roots. Reproduction of the nematode proceeds throughout the winter and considerable root breakdown is in evidence towards the end of the season.

During 1956-57 field trials were set out to determine the status of *M. hapla* as a pest, and laboratory and field work concerning control was undertaken.

II. EFFECT OF RUNNER INFESTATION ON SUBSEQUENT PERFORMANCE.

In 1956 a field trial was carried out at Ormiston to determine whether root-knot nematode infestations on planting material affected the yielding ability of the plants. The soil was a latersol.

The planting material was dug from an infested runner bed and graded into the following classes on the basis of galling:—

Heavy.—Galls on more than half the fibrous roots.

Moderate.—A light scattering of galls throughout the root system.

Nematode-free runners were produced from the same area by allowing the runners to root in a 6 in. mulch of sawdust spread between the parent plants before runnering commenced.

The layout was a 6 x 4 randomised block with 2 double rows and 30 datum plants per plot. Soil fumigation was carried out on Mar. 5, 1956, by injecting ethylene dibromide ($12\frac{1}{2}$ per cent. v/v formulated from a concentrate with S.G. $\frac{25}{25}$ 2·170) in holes 6 in. deep and 1 ft. apart under the intended planting lines two weeks prior to planting at a rate of 2 ml. per hole.

Berries were graded as follows:—

Punnet berries.—6 or fewer across a punnet 4 in. wide.

Factory berries.—More than 6 across a punnet 4 in. wide.

After harvesting, the plants in one row of each double row were dug and the roots rated as 0, 1, 2, 3, or 4 corresponding to increasing severity of root-knot nematode infestation.

Results and treatments are presented in Tables 1 and 2.

Table 1.

Effect of Relative Nematode Infestation of Planting Material on Yields.

Fumigation Treatment.	Relative Nematode Infestation.	Yields per Plot (g.).		
		June–July.	Oct.–Nov.	Total.
Nil ..	Heavy	226	2,212	4,511
Nil ..	Moderate	600	3,405	7,948
Nil ..	Nil	938	3,478	8,099
EDB ..	Heavy	364	2,655	6,091
EDB ..	Moderate	566	3,435	8,296
EDB ..	Nil	1,053	3,925	9,071
Differences necessary for significance	5%	234	623	1,691
	1%	324	861	2,338

Table 2.

Effect of Relative Nematode Infestation of Planting Material on Berry Size and Gall Development.

Fumigation Treatment.	Relative Nematode Infestation.	Mean Root-knot Rating.	Number of Punnet Berries per 100 Factory Berries.
Nil	Heavy	4·0	174
Nil	Moderate	3·9	151
Nil	Nil	2·2	154
EDB	Heavy	4·0	174
EDB	Moderate	3·8	171
EDB	Nil	1·5	174
Differences necessary for significance	5%	0·57	No significant differences
	1%	0·79	

III. CONTROL IN RUNNERS BY HOT WATER TREATMENT.

Hot water treatment of strawberry propagation stock has been recommended overseas as a method of controlling stem and bulb eelworm, *Ditylenchus dipsaci* (Kühn), the foliar nematodes *Aphelenchoides ritzema-bosi* (Schwartz) and *A. fragariae* (Ritzema-bos), the root-knot nematode *Meloidogyne hapla* Chitwood, and the root-lesion nematode, *Pratylenchus penetrans* (Cobb) (Staniland 1953; Goheen and McGrew 1954; Goheen, McGrew and Smith, 1956). A number of different temperature-time combinations have been suggested for the treatment of dormant plants.

This project involved the determination of lethal temperature-time combinations for egg masses of *M. hapla* attached to strawberry roots, and a study of the effect of such combinations on treated runners.

Table 3.

Effect of Hot Water Treatment on Plant Survival and Nematode Development.

Temperature (°C.).	Immersion Time (min.).	No. of Plants Surviving.	No. of Plants with Galls.	Mean Root-knot Rating.
46	8	40	28	1·5
	10	37	25	1·7
	12	36	15	0·6
	14	37	16	0·7
	16	38	12	0·5
47	8	36	20	1·1
	10	39	20	1·1
	12	37	15	0·7
	14	38	16	0·9
	16	37	16	0·7
48	6	40	21	0·7
	8	38	11	0·4
	10	40	6	0·2
	12	39	3	0·1
	14	38	6	0·2
49	4	39	11	0·6
	6	38	8	0·4
	8	38	11	0·5
	10	39	5	0·2
	12	31	2	0·1
50	2	40	12	0·4
	4	38	12	0·5
	6	38	12	0·7
	8	36	15	0·8
	10	32	8	0·5
Untreated		40	40	3·75

Lethal temperature-time combinations for egg-masses of *M. hapla* were determined by treating infested roots in a 3 gal. container of water over a heating source. The roots were suspended in a wire basket and the water was agitated during treatment. Immediately after treatment the roots were plunged into water at room temperature. Five large egg masses were removed from each lot of treated roots, placed in 4 ml. of distilled water and incubated for 2 weeks at room temperature.

The effect of hot water treatment on the runners was determined by treating infested runners in a similar manner and planting in field plots. The plants were entirely immersed during treatment. There were four replicates of each 10-plant treatment. Runners were treated in March 1956 and examined for nematode infestation in October 1956.

The results are presented in Table 3.

IV. EFFECT OF HOT WATER TREATMENT OF RUNNERS ON YIELD.

During 1957 runners were dug from an infested plot and graded in the field for the purpose of the experiment. One series "field selected" had few, if any, galls. Runners with galls on at least half the fibrous roots were considered as "heavily infested" and used for hot water treatments. The treatments were applied in a manner similar to that mentioned previously.

A 7 x 4 randomised block layout was used with 2 double rows containing 40 datum plants per plot. The site, which was adjacent to that used for the 1956 trial, was treated on Mar. 4, 1957, with EDB (12½ per cent. v/v) at the rate of 20 gal. per acre. The trial was planted on Mar. 18, 1957.

After harvesting, the plants in one of each of the double rows were dug and rated for nematode infestation. As an indication of plant size, the number of plantlets in each crown was recorded.

The results are presented in Table 4.

Table 4.

Effects of Hot Water Treatment of Runners on Growth, Yield and Nematode Development.

Treatment.	No. of Plants per Plot.	Weight of Berries per Plot (g.).	Mean Root-knot Rating.	No. of Plantlets per Crown.
Heavily infested runners	35·75	6,858	3·6	3·30
Field selected runners	37·25	9,507	2·9	3·85
Runners from sawdust mulch ..	37·50	11,861	2·1	4·88
Hot water 47°C. 2 min.	36·75	8,178	3·6	3·75
Hot water 47°C. 6 min.	35·25	8,622	2·7	4·28
Hot water 47°C. 10 min.	33·0	8,242	2·1	4·20
Hot water 47°C. 14 min.	31·25	6,707	1·7	4·12
Differences necessary for significance { 5%	2·78	1,895	0·42	0·62
Differences necessary for significance { 1%	3·81	2,597	0·58	0·84

V. CONTROL IN PLANTING MATERIAL WITH CHEMICALS.

Preliminary tests of ovicides were carried out in the laboratory by immersing egg masses for one hour in concentrations of each chemical ranging from 0·0125 per cent. to 4·0 per cent. After washing the egg masses in distilled water and incubating for four days at room temperature, the minimum concentrations for complete inhibition of hatching were determined. The following five ovicides were then selected for further investigations:—chlorophenyl mercury chloroglucine (''Aaventa''), phenyl mercury dinapthylmethane disulphonate (''Phenyl mercury fixtan''), phenyl mercury salicylate, iodine and mercuric chloride.

Nematode-infested runners were held with crowns and roots in solutions of these materials for one hour. The concentrations were based on the results of the preliminary laboratory screening, and 4 ml. of detergent (''Teepol'') were added to each litre of solution to assist in penetration of the egg masses protruding from the roots. After removal from the chemical, excess liquid was

Table 5.

Survival and Root-knot Ratings of Runners Treated in Chemical Dips.

Treatment.	Dip Conc. (%).	No. of Plants Surviving (40 per treatment).	Mean Root-knot Rating.
"Aaventa" (chlorophenyl mercury chloroglucine)* ..	1·0	25	0·5
	0·5	23	1·0
	0·25	24	1·0
	0·125	34	1·7
"Phenyl mercury fixtan" (phenyl mercury dinaphthyl-methane disulphonate)*	1·0	4	0·2
	0·5	14	0·4
	0·25	21	1·1
	0·125	26	1·4
Phenyl mercury salicylate	0·1	3	0
	0·05	16	1·0
	0·025	25	1·5
	0·0125	26	1·3
Iodine	0·5	3	2·7
	0·25	9	2·8
	0·125	15	2·5
	0·06	27	2·9
Mercuric chloride	0·25	4	0
	0·125	16	0·2
	0·06	33	0·4
	0·03	31	0·5
Control—No treatment means of 4 replicates ..		32	2·9

* Concentrations of dip : refer to formulation, not active ingredient.

drained off and the runners were planted in field plots which had been fumigated with methyl bromide at the rate of 1 lb. per 80 sq. ft. There were 10 plants per treatment and 4 replications. Treatments were applied in April 1957 and data on survival and gall development were collected in August 1957.

Results are presented in Table 5.

VI. DISCUSSION.

The deleterious effect of nematode infestation of strawberry runners on subsequent growth and yield is evident from the results of the two field trials (Tables 1, 2 and 4). In the first trial an interesting feature is the much heavier June-July crop from nematode-free runners than from the moderately infested runners. This early crop commands a higher market price than later fruit. It is obvious from these figures that selection of planting material can be expected to increase strawberry yields.

Numerous temperature-time combinations for hot water treatment will control *M. hapla* on infested runners. Treatment at 47 deg. C. for 6, 10 and 14 min. did not increase yield following nematode control and this is apparently due to the early setback caused by root injury. At the end of the season the treated plants were larger than the untreated runners.

Chlorophenyl mercury chloroglucine, phenyl mercury dinaphthylmethane disulphonate, phenyl mercury salicylate and mercuric chloride dips controlled *M. hapla* but killed many of the infested roots.

The highest yields were produced by strawberry runners which had rooted in 6 in. of sawdust spread around the parent plants. In addition to relative freedom from nematodes, these plants were less etiolated than normally rooted runners.

No plant death was attributable to heavy nematode infestation.

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MILD STRAIN PROTECTION AS A MEANS OF REDUCING LOSSES FROM THE QUEENSLAND WOODINESS VIRUS IN THE PASSION VINE.

By J. H. SIMMONDS, M.Sc., Government Plant Pathologist,
Division of Plant Industry.

SUMMARY.

The need for a means of reducing loss from the woodiness disease of passion vine (Passiflora edulis Sims) is briefly stated and a practical attempt to achieve this object by making use of mild strain protection is described.

In replicated trials in their second and third years, vines carrying a mild strain of the woodiness virus showed a marked superiority over those carrying a severe strain in number, weight and size of fruit free from woody symptoms.

I. INTRODUCTION.

Diseases of one sort or another have always made passion vine growing in Queensland an uncertain venture. In the early days brown spot was serious; later, woodiness came into prominence, with Fusarium wilt important in more restricted localities. Satisfactory control measures have been devised for brown spot and wilt (Simmonds 1930; Purss 1958) but woodiness is still a limiting factor. Fig. 1 shows various stages of severity of the disorder.

Woodiness was first recorded in Queensland in August 1927. At that time the disease was of rare occurrence and vines could be expected to remain in production for eight years or more. By 1932 woodiness had become comparatively common and it was not long before the expected commercial life of a plantation had dropped to as low as two years.

II. THE WOODINESS VIRUS IN QUEENSLAND.

In 1928 R. J. Noble described a woodiness disease of the passion vine in New South Wales which he showed was due to a sap transmissible virus (Noble 1928). This disease was further described in 1938 (Noble and Noble 1938) and shown to be transmissible by four species of aphids. Later Magee (1948) stated the virus concerned to be cucumber virus 1.

Because of the similarity of symptoms it has been generally assumed that the disease in Queensland is the same as that described for New South Wales. However, McKnight (1953) was unable to produce any evidence that the virus causing the Queensland disease was cucumber mosaic.

The position has recently been clarified by Taylor (1959), who showed that two distinct viruses cause woody symptoms in passion fruit. The disease occurring in Victoria is caused by a strain of cucumber mosaic virus, while the Queensland disease is due to a distinct and so far unidentified virus. The

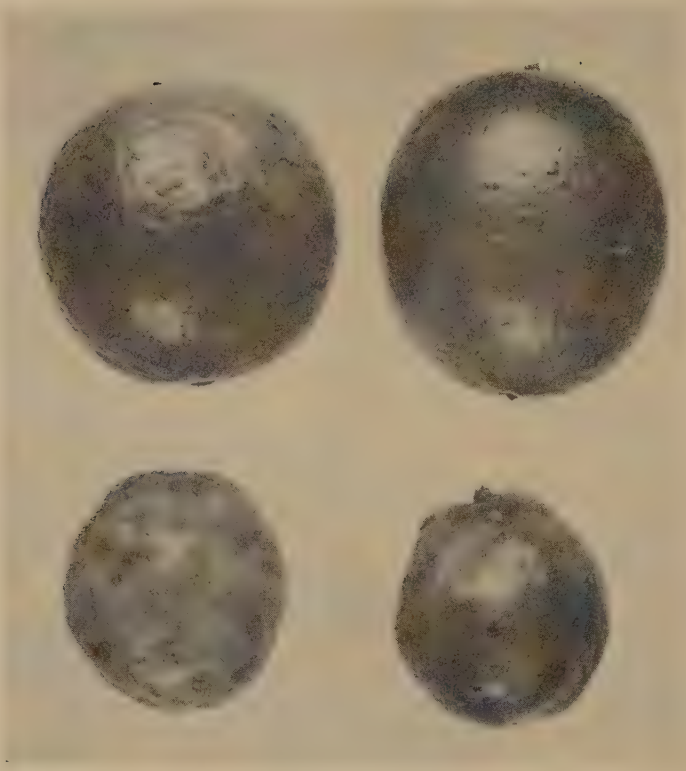

Fig. 1.

Passion Fruit Woodiness. Above: Severe and mild form of the pimpled condition. Below: Severe woody symptoms.

production of "woody" symptoms in the rind of passion fruit is apparently a common reaction to more than one stimulus. For example, fruit fly stings will produce an appearance difficult at times to distinguish from virus effects.

The Victorian woodiness disease has been known in Queensland on imported nursery plants consisting of *Passiflora edulis* scions grafted on to *P. caerulea* stock. It is characterised by a broad yellow or yellow brown mosaic of the leaves, which are markedly deformed, while the whole vine remains stunted almost to a cessation of growth. So far there has been no evidence of the Victorian virus infecting *P. edulis* plants raised in Queensland. It is likely that the Queensland woodiness disease occurs also in New South Wales, at least in the northern part of that State.

III. MILD STRAIN PROTECTION AS A POSSIBILITY.

There is no evidence of any worthwhile resistance to woodiness disease in individuals of *P. edulis* and the fact that infection frequently comes from outside the plantation (McKnight 1953) gives little hope for control through the vector.

McKnight (1953) put forward a strain complex hypothesis for the Queensland woodiness virus and suggested the possibility of obtaining mild strain immunity.

The theory of strain protection whereby two strains of the same virus are mutually exclusive once they have colonised a host is now a recognised tool for determining the relationship of viruses affecting a common host (Price 1940). However, the use of this theory in reducing economic losses from virus diseases has been by no means so successful. For a short-term crop the work involved is seldom justified, while for a long-term perennial one there is the risk that a severe strain will eventually superimpose itself on the milder form or that complications will arise over reactions with other viruses (Posnette and Todd 1955; Grant and Higgins 1957). The passion vine lies between these two extremes. A cropping cycle of three to four years would be economically sound and an alteration to the virus complement after this time of less importance. In an endemic area, too, natural infection is so rapid and universal that there can be few qualms about deliberately infecting the vines with a mild form of the disease.

The work described below was essentially a practical attempt to apply the mild strain protection theory to obtaining relief from the ravages of woodiness. Many interesting aspects of the fundamental relationship between the strains of the Queensland woodiness virus and its host remain to be investigated.

(1) Selection of Mild Strains.

For the preliminary collection of virus strains, plant material was obtained in three commercial plantations in south-eastern Queensland from individual vines which appeared to have some distinct symptoms associated with their virus complement. Transfers to potted seedlings were made in the glasshouse, using four methods. The majority were made by the petiole graft or tissue insertion method described by McKnight (1953). Sap transmission and tip grafts were used to a lesser extent. A few transmissions were made from *P. edulis* var. *flavicarpa*, *P. laurifolia* and a *P. edulis* hybrid in addition to those from the purple passion vine.

Fifty plants were inoculated in this way on May 5 and July 31, 1953. Of these 32 were planted in the field at the Redlands Experiment Station on Sept. 17. Periodical recordings were made of the virus symptoms displayed until the first crop of fruit matured in October 1954. Unfortunately, an unexpected visitation of Fusarium wilt caused the death of a number of vines before maturity.

Reviewing the observations made, there seemed little definite correlation between the origin and symptoms displayed by the graft material selected and the type of symptoms developed subsequently by the grafted plant. At the time the latter were fruiting it was observed that severe bullate mottle was associated with moderate to excessive numbers of woody fruit, whereas in the few cases where exceptionally few woody fruit developed, the bullate mottle was inconspicuous and the virus symptoms in general more moderate. In one vine which displayed almost pure fern-leaf symptoms, woody fruit were in excess.

This project was carried a step further in 1954, when from the trial just described three plants were selected which produced a much below average number of woody fruit and had a milder virus record generally. Leaf and tip material from these was grafted on to *P. edulis* seedlings in the glasshouse on Sept. 30 and Oct. 21, 1954. At the same time graft transmissions were made from two plants naturally infected with woodiness.

This material was used for a 6 x 3 replicated field trial planted in Border F7 at the Redlands Experiment Station. The mild strains used were 443P, 443S and 523D and the normal strains C8 and P3. One treatment without prior inoculation was included.

Although the trial was planted in new land where it was hoped Fusarium wilt would not be a factor, so many vines were affected by this disease that statistical analyses was impossible. However, a count of woody fruit in the spring crop harvested on Nov. 25, 1955, was definitely in favour of the mild strains, there being fewer than half the number of woody fruit in these as in the vines inoculated with the normal strain.

Fortunately, about this time a means of overcoming the wilt problem through the use of resistant stocks became available (Purss 1958). The opportunity was taken to incorporate some of the vines bearing mild strains as scions in a stock-scion trial laid down in Border C6 in 1955 and further observations on their cropping capacity were made.

(2) Replicated Field Trials, 1956 and 1957.

Early in 1956 tips from selected vines carrying mild strains and one with the normal severe strain were tip and side grafted onto a range of Fusarium-resistant stocks then available. On Mar. 28, 1956, these were planted in Border F7 at the Redlands Experiment Station. Two series infected with mild strains of woodiness (10 and 15) were compared with a normal woody strain (16) on two types of stock (*P. edulis* var. *flavicarpa* and a *P. edulis* hybrid). Each stock-scion combination was replicated three times. As the stocks appeared to have no influence on the amount of woodiness developed, the harvest figures were subsequently analysed as a 3 x 6 trial without reference to the stock factor.

The two mild strains originated from 443P and comprised "10" from plant 10 in the 1955 stock-scion trial Border C6 and "15" from plant 15E

Table 1.

Replicated Trial, 1956. Mean Percentage Fruit per Vine, Spring Crop, 1956.

Virus Strain.	Non-woody.	Woody.
10 (mild)	95·7	4·3
15 (mild)	81·0	19·0
16 (severe)	32·0	68·0

Table 2.

Replicated Trial, 1956. Mean Number and Weight of Fruit per Vine, Spring Crop, 1957.

Virus Strain.	Number		Weight (lb.).	
	Non-woody.	Woody.	Non-woody.	Woody.
10 (mild)	501	114	41·3	7·8
15 (mild)	453	122	33·6	8·3
16 (severe)	103	373	6·7	22·2

in the 1954 replicated field trial in Border F7. The severe strain "16" originated from P3 and was obtained from plant 16E in the latter trial.

A light spring crop was harvested in 1956 and normal crops in 1957 and 1958. The fruit were mostly mature green when picked. They were hand-graded for size and the presence of woodiness symptoms. A small number of fruit exhibiting a mild pimpled condition of the skin (Fig. 1) of no commercial significance was classed with the non-woody. Results appear in Tables 1–4 and are illustrated in Figs. 2–5.

Table 3.

Replicated Trial, 1956. Mean Number and Weight of Fruit per Vine, Spring Crop, 1958.

Virus Strain.	Number			Weight (lb.).		
	Large.	Small.	Woody.	Large.	Small.	Woody.
10 (mild)	242	289	81	20·4	16·2	4·8
15 (mild)	195	267	98	15·4	14·3	5·4
16 (severe)	89	147	270	7·0	8·0	12·9

As a result of the method of propagating the virus (at first by leaf insertion and later by tip grafting), the basic *P. edulis* plant material had been largely restricted to two lines. The inclusion of some additional material seemed desirable to ensure that the results obtained were not linked with host reaction. Accordingly, a further trial was laid down in 1957 along the lines of that of the previous year but including one treatment in which tips from

Fig. 2.

Field Trial, 1956. Spring Crop, 1957. Vine No. 5, Mild Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

Fig. 3.

Field Trial, 1956. Spring Crop, 1957. Vine No. 4, Severe Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

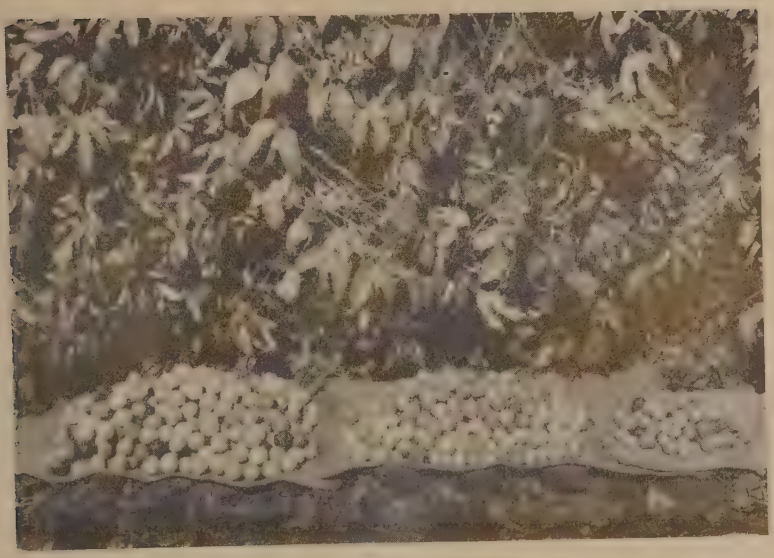

Fig. 4.

Field Trial, 1956. Spring Crop, 1958. Vine No. 5, Mild Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

Fig. 5.

Field Trial, 1956. Spring Crop, 1958. Vine No. 4, Severe Strain. Left: Non-woody, large. Middle: Non-woody, small. Right: Woody.

Table 4.

REPLICATED TRIAL, 1956. SUMMARY-MEAN VALUES AND SIGNIFICANT DIFFERENCES, SPRING CROP, 1958.

Virus Strain.	Total Number of Fruit.		Percentage Large.		Percentage Woody.	
	Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.
A. 10 (mild)	2·76	573	38·3	38·4	20·7	12·5
B. 15 (mild)	2·71	513	36·3	35·0	23·8	16·2
C. 16 (severe)	2·69	493	24·4	17·1	46·7	53·0
S.E. mean of 6 ..	·070 No significant differences		1·23 A, B >> C		1·63 A, B << C	

Prior to analysis, the log transformation was used on Total Number and the inverse sine transformation was used on Percentage Large and Percentage Woody in order to equalise the variances.

seedlings grown from commercial seed were tip-grafted onto flavicarpa-edulis combinations already infected with mild strain virus. The mild strain used was "10" and was obtained from the original plant 10 in the stock-scion trial and vine 5 in the 1956 replicated trial. The grafted plants were set out alongside the previous 1956 trial on Aug. 8, 1957. The first crop was harvested in November 1958. The results appear in Tables 5 and 6.

Table 5.

REPLICATED TRIAL, 1957. MEAN NUMBER AND WEIGHT OF FRUIT PER VINE, SPRING CROP, 1958.

Virus Strain.	Number.			Weight (lb.).		
	Large.	Small.	Woody.	Large.	Small.	Woody.
Mild	168	171	39	15·0	10·2	2·4
Mild (commercial scion) ..	196	192	43	15·8	11·0	2·7
Severe	19	156	265	1·5	8·6	12·9

Table 6.

REPLICATED TRIAL, 1957. SUMMARY—MEAN VALUES AND SIGNIFICANT DIFERRENCES, SPRING CROP, 1958.

Virus Strain.	Total Number.		Percentage Large Fruit.		Percentage Woody Fruit.	
	Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.	Trans. Mean.	Equiv. Mean.
A. Mild	2·54	350	40·1	41·5	19·6	11·3
B. Mild (commercial scion) ..	2·61	408	40·4	42·0	18·8	10·4
C. Severe	2·62	412	11·2	3·7	51·6	61·5
S.E. Scion mean	·044 No significant differences		1·58 A, B >> C		2·09 A, B << C	

Prior to analysis the log transformation was used on Total Number and the inverse sine transformation was used on Percentage Large Fruit and Percentage Woody Fruit in order to equalise the variances.

Periodic vine-to-vine examinations for leaf symptoms in the 1956 and 1957 field trials showed that in addition to the increased number of woody fruit the vines infected with a severe strain in general exhibited a more pronounced chlorotic mottle and bullate mottle than those bearing a mild strain.

IV. DISCUSSION.

It will be seen from the tables that it is possible to inoculate passion vines with selected strains of the Queensland woodiness virus which will protect them under natural conditions of infection from more severe strains. The mild strain has apparently remained uncontaminated through successive transfers over five years.

In the replicated field trials, vines bearing severe strains were interspersed among those inoculated with the mild form. Owing to close planting, much interweaving of runners took place. Although reasonable precautions were taken during pruning operations, opportunity for mechanical transfer must have occurred. In spite of this no instance was noted of a vine showing symptoms suggesting invasion by the severe strain. Differences from vine to vine in yield and proportion of woody fruit occurred throughout the experiments but the variation remained within the mild or severe category, depending on the nature of origin.

It is becoming increasingly necessary in south-eastern Queensland to work passion vines onto a Fusarium-resistant stock. This makes the use of mild strain protection a practical proposition as the only additional work entailed is the selection of scion material from a parent vine bearing the required strain of the woodiness virus.

In this investigation, because little was known regarding possible strain selection by mechanical transfer and because severe woody symptoms are at a minimum during warm weather, propagation has been by tip-grafting during the summer months. Mechanical sap transfer at this or other times might yield equally satisfactory results and eliminate the need for grafting where wilt was not a problem.

A demonstration plot using both methods has now been planted and it remains to be seen how far commercial practice is prepared to accept the avenue for control suggested by these investigations.

V. ACKNOWLEDGEMENTS.

Grateful acknowledgement is made to the staff of the Redlands Experiment Station for assistance in cultural operations connected with the field trials and to Mr. P. McGovern, Senior Biometrician, for statistical analyses and advice.

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STUDIES OF THE COCCOIDEA. 10. NEW SPECIES OF DIASPIDIDAE.

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SUMMARY.

New species of scale insects from Queensland described in the family Diaspididae are Ancepaspis longicauda, A. magnicauda, A. asperata, A. reticulata, A. rotundicauda, A. striata, Andaspis numerata, Aulacaspis australis, Doriopus bilobus, Phenacaspis difissata, Poliaspis attenuata, **and** P. elongata. **The genus** Doriopus **is also new.**

Subfamily PHOENICOCOCCINAE.

Ancepaspis longicauda new species.

(Figs. 1 and 2)

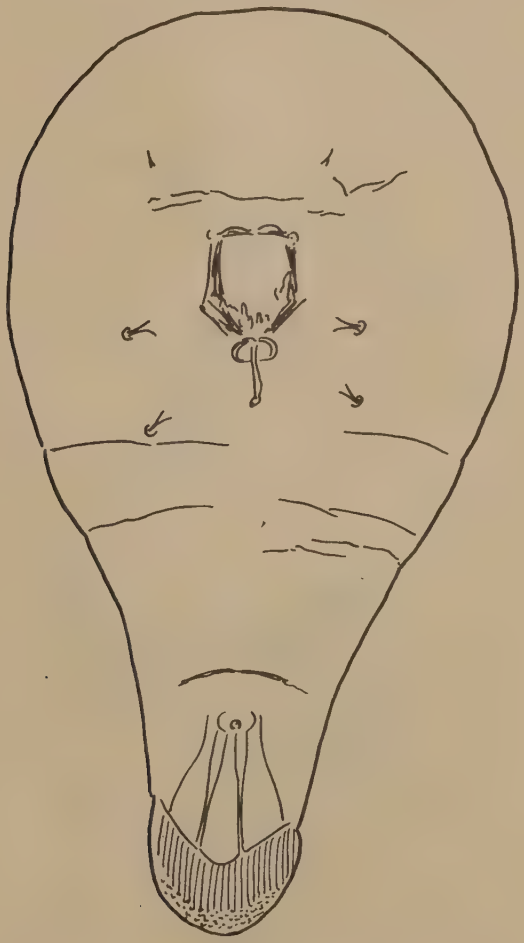

Fig. 1.

Ancepaspis longicauda. Outline of Adult Female, X 100.

Locality and host.—Queensland: Marmor, on *Casuarina glauca* Sieb., Oct. 1955.

Habit.—Insects occurring singly or in small groups in forks of branchlets, or singly in crevices of the rough bark on small branches.

Recognition characters.—Adult female pyriform, mostly membranous; length of slide specimen 1·3 mm., width 0·5 mm. Pygidium long, sclerotized, anterior part broad, posterior part or cauda mostly densely sclerotized; caudal extension wide, broadly crescentic, dorsally reticulate, apex broadly rounded, entire. Ventral precaudal area with three longitudinal furrows, one

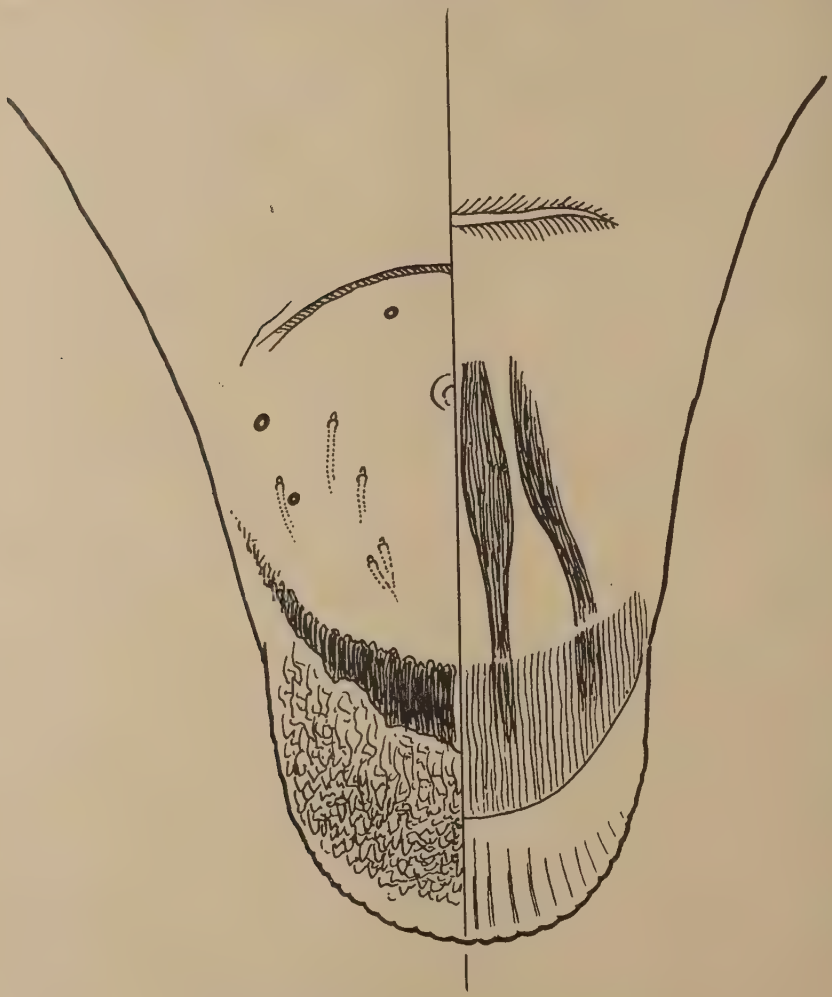

Fig. 2.

Ancepaspis longicauda. **Enlargement of Pygidium, X 380.**

median and a submedian pair, all anteriorly narrow and widening posteriorly, intervening dense longitudinal bands reaching slightly anterior to the position of the anal opening. Vulva anterior to the anal opening, surrounded by a circular fold. Dorsal ducts long, slender, moderately numerous, opening on the cauda. Spiracles large, anterior pair with associated pores.

Notes.—The shape of this species is proportionately longer than that shown by the species described by Ferris (1942), of which only *A. edentata* has an entire caudal margin. *A. longicauda* differs from this species in having a long densely sclerotized posterior caudal portion, and not having an anteriorly arched thickened dorsal ridge.

Type Reg. No. T5768 and paratype No. T5769 in the Queensland Museum.

Ancepaspis magnicauda new species.

(Figs 3 and 4.)

Fig. 3.

Ancepaspis magnicauda. Outline of Adult Female, X 130.

Locality and host.—Queensland: Tara, on *Acacia harpophylla* F. Muell. ex Benth., Aug. 1957 (L. Pedley).

Habit.—Insects mostly single in cracks, crevices and bark scars on twigs and small branches.

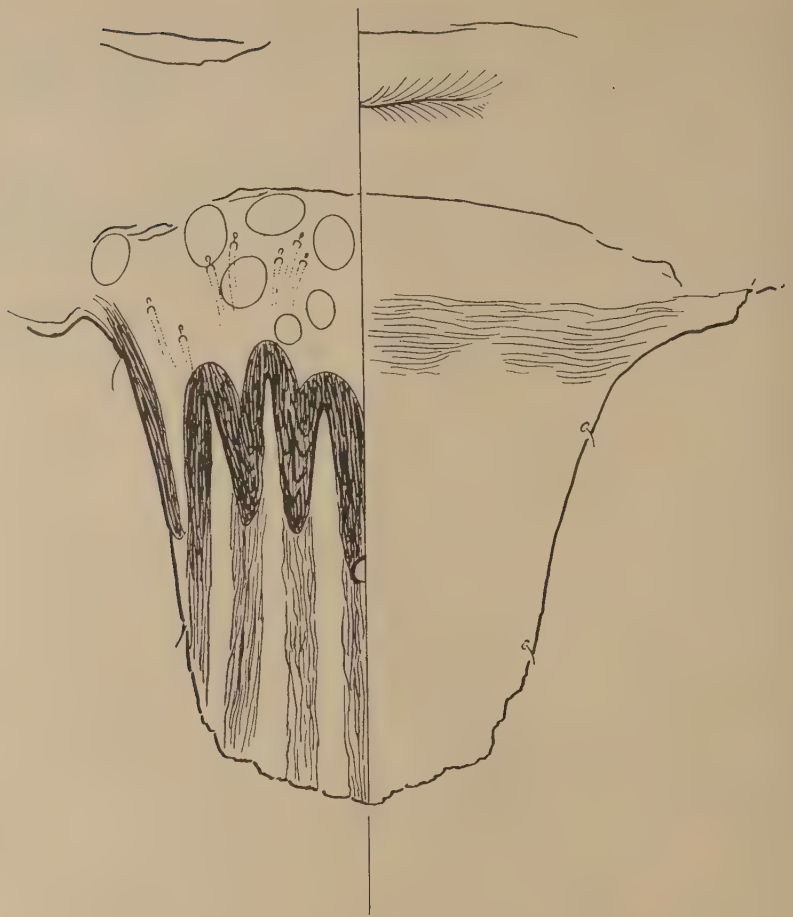

Fig. 4.

Ancepaspis magnicauda. Enlargement of Pygidium, X 330.

Recognition characters.—Adult female membranous, globular, sub-circular, with the cauda extending beyond the general body outline; length of slide specimen 0·8 mm., width 0·7 mm. Pygidium anteriorly membranous, basally broad, cauda slightly more than half pygidial width; precaudal portion lightly sclerotized but with many subcircular less dense areas. Cauda as wide as long, basally densely sclerotized in a scalloped border; posteriorly with seven longitudinal bars of denser sclerotization symmetrically placed; posterior margin with some small indentations. Anal opening obscured by the dense sclerotization. Vulva in anterior membranous part of pygidium. Ducts sparse, long and slender. The four spiracles close and embodied in a subquadrate area of sclerotization. Anterior spiracles with associated pores.

Notes.—The median longitudinal dense caudal bar may appear as doubled for part of its length. This species resembles *A. novemdentata* Ferris but the caudal apex is not deeply fissured, and the spiracles are close and embodied in a subquadrate sclerotized plate.

Type Reg. No. T5770 and paratype No. T5771 in the Queensland Museum.

Ancepaspis asperata new species.

(Figs. 5 and 6.)

Locality and host.—Queensland: Tugun, on *Casuarina littoralis* Salisb., May 1953.

Habit.—Insects mostly single under rough pieces of bark or in forks of small branches.

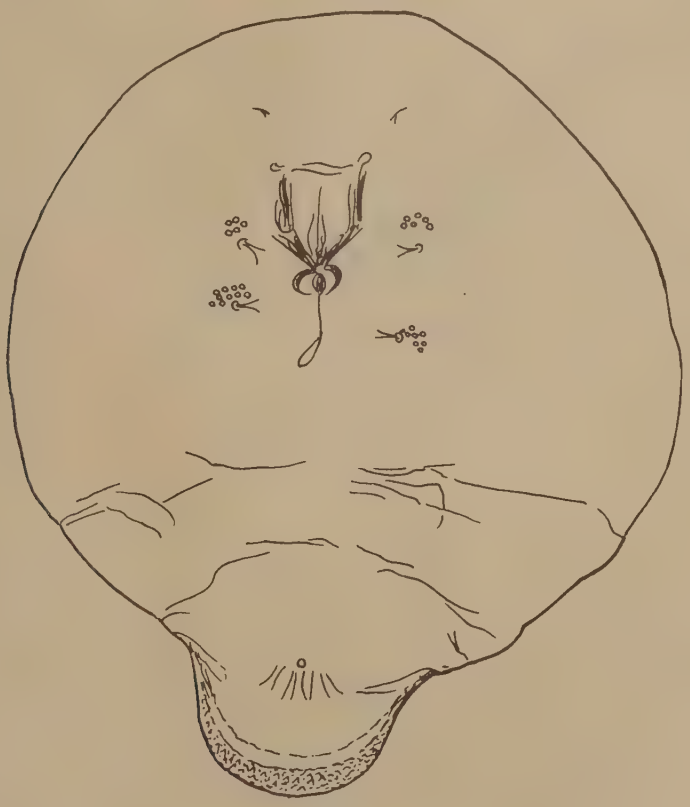

Fig. 5.

Ancepaspis asperata. Outline of Adult Female, X 200.

Recognition characters.—Adult female membranous, globular; length of slide specimen 0·6 mm., width 0·5 mm. Pygidium subcircular, anteriorly not differentiated by a thickened ridge. Densely sclerotized caudal portion crescentic or semicircular with diverging lateral arms. Precaudal median and submedian longitudinal furrows short, intervening dense bands short and oval, precaudal submarginal dense areas with mesal margin curved. Densely sclerotized caudal dorsal surface with irregularly crescentic rows of asperities. Dorsal ducts sparse, slender. Anal opening centrally in pygidium. Anterior spiracles with five to seven associated pores, posterior spiracles with nine to 11 associated pores.

Fig. 6.

Ancepaspis asperata. Enlargement of Pygidium, X 330.

Notes.—The apical margin of the cauda may have symmetrically placed indentations. In some specimens the cauda may be narrowly crescentic, in other specimens it may be almost quadrate. The number of pores associated with the spiracles may be fewer in number.

This species resembles *A. edentata* (Ferris) but differs in not having on the pygidium the anterior arched ridge and in having the longitudinal furrows and bands in the precaudal region. There are some resemblances in pygidial characters to *A. longicauda* but in the latter species the cauda is longer, the sclerotized deposition is reticulate and there are no marginal indentations. In addition, *A. longicauda* is more elongate in shape and the posterior spiracles are without associated pores.

Type Reg. No. T5776 and paratype No. T5777 in the Queensland Museum.

Ancepaspis reticulata new species.

(Figs. 7 and 8.)

Locality and host.—Queensland: Pikedale, on *Casuarina luehmanni* R. T. Baker, Oct. 1954.

Habit.—Insects under rough pieces of bark tissue or in forks of small branches.

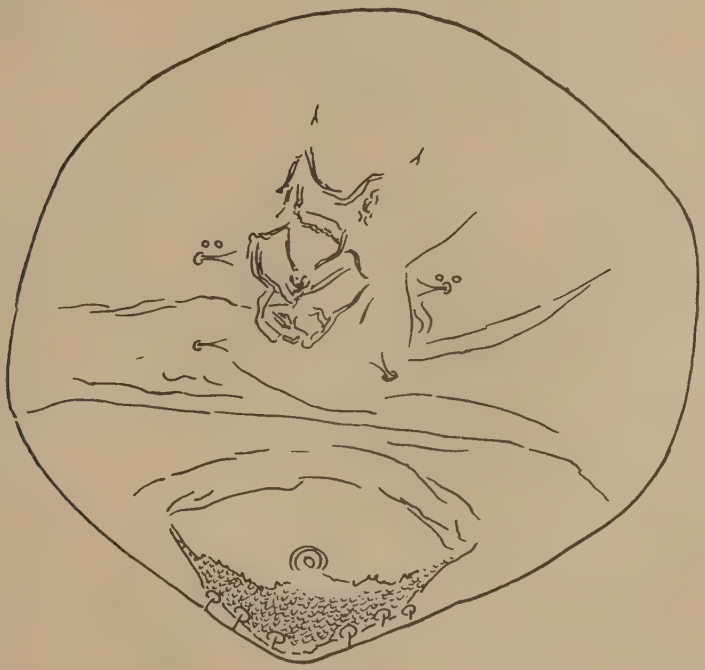

Fig. 7.

Ancepaspis reticulata. Outline of Adult Female, X 160.

Recognition characters.—Adult female globular, membranous; length of slide specimen 0·5 mm., width 0·5 mm. Pygidium large and mostly sclerotized. Densely sclerotized cauda crescentic, lateral arms anteriorly joined by a dorsal arched ridge of sclerotization. Ventral surface with median and submedian furrows; intervening dense longitudinal bands elongate-oval, the submarginal dense area with inner margin curved. Cauda dorsally with asperities, segmental marginal dorsal spines large with bases surrounded by less densely sclerotized areas, giving the margin a scalloped appearance. Anal opening posteriorly placed. Anterior spiracles with associated pores.

Notes.—The shape of most of the specimens examined is irregularly globular, although in some instances the outline is more uniform and almost subcircular.

The species has some resemblance to *A. asperata* but the large spines on the cauda and the demarcated spine bases distinguish the species.

Type Reg. No. T5778 and paratype No. T5779 in the Queensland Museum.

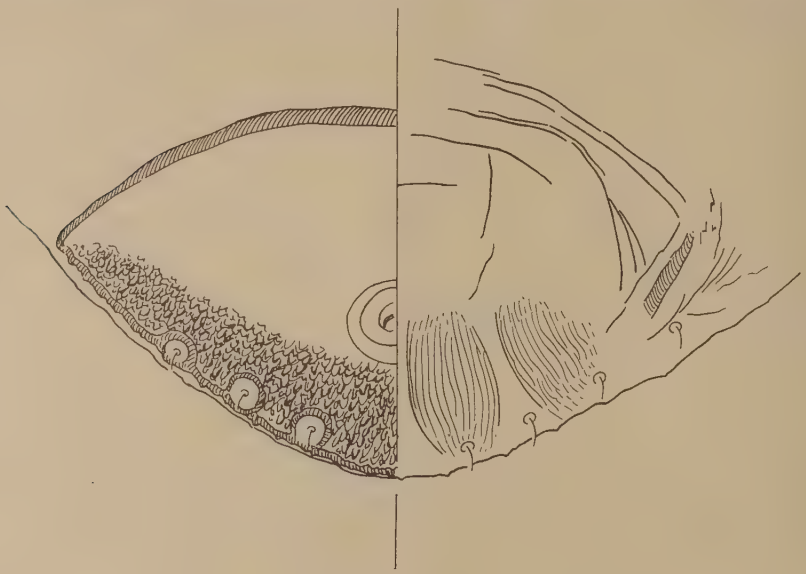

Fig. 8.
Ancepaspis reticulata. Enlargement of Pygidium, X 270.

Ancepaspis rotundicauda new species.

(Figs. 9 and 10.)

Locality and host.—Queensland: Moggill, on *Casuarina cunninghamiana* Miq., Sept. 1943.

Habit.—Insects single or in small groups in crevices and scars on small branches and in forks of the branchlets, accompanied by small amounts of mealy material.

Recognition characters.—Adult female globular, membranous; length of slide specimen 0·5 mm., width 0·4 mm. Pygidium more or less round, densely sclerotized cauda crescentic, arms anteriorly joined by a dorsal arched ridge. Ventral precaudal area with a median and a pair of submedian furrows; the intervening dense bands elongate, widest posteriorly; the submarginal areas

Fig. 9.

Ancepaspis rotundicauda. Outline of Adult Female, X 190.

with inner and posterior margins angled. Ducts sparse, long and slender. Anal opening small, centrally placed on pygidium. Vulva anteriorly on pygidium. Anterior spiracles with two or three associated pores; posterior spiracles without associated pores.

Notes.—The pores associated with the anterior spiracles may vary from one to five. The crescentic cauda may appear to be rolled ventrally on the posterior margin. The dorsal anteriorly arched ridge on the pygidium may be semicircular, giving the cauda and ridge a round shape, or the ridge may be broadly arched, giving the cauda and ridge a semicircular shape.

This species has some resemblance to *A. asperata* but differs in that the posterior spiracles have no associated pores and the pygidium has the anteriorly arched dorsal ridge.

Type Reg. No. T5780 and paratype No. T5781 in the Queensland Museum.

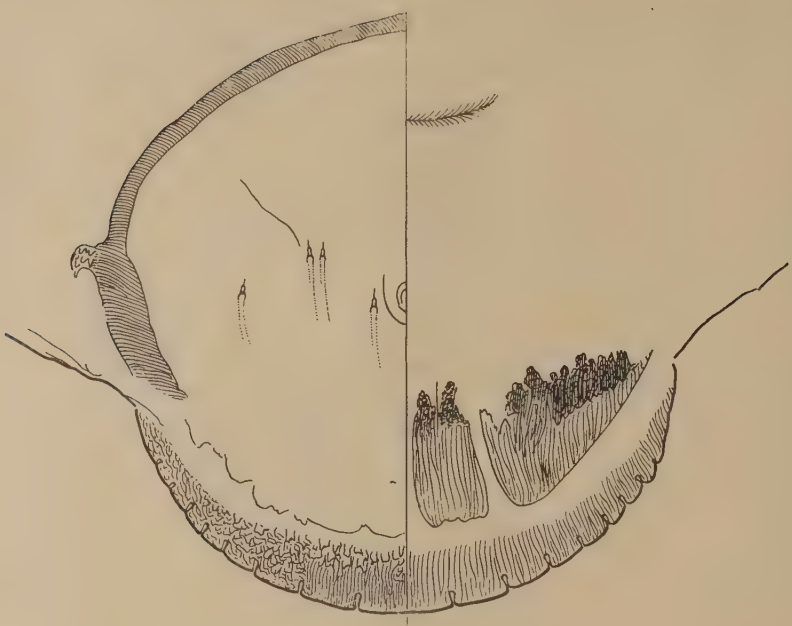

Fig. 10.

Ancepaspis rotundicauda. Enlargement of Pygidium, X 260.

Ancepaspis striata new species.

(Figs. 11 and 12.)

Locality and host.—Queensland: Yelarbon, on *Casuarina lepidophloia* F. Muell., Sept. 1947.

Habit.—Insects occurring under loose bark fragments, in scars and in forks of branchlets.

Recognition characters.—Adult female elongate-oval, membranous except pygidium; length of slide specimen 0·85 mm., width 0·4 mm. Pygidium large, mostly sclerotized. Cauda large, crescentic, with four symmetrically placed longitudinal denser sclerotized bands, apex rounded with the bands projecting as small marginal round protuberances. Precaudal area sclerotized, anteriorly

bordered dorsally by a semicircular arch joining the arms of the caudal crescent. Anterior border of cauda scalloped. Dorsal ducts long and slender. Anal opening small, oval, placed anteriorly in pygidium. Pygidial segmental spines submarginal on caudal denser sclerotized bands. Vulva in a semicircular ventral fold. Anterior spiracles with six or seven associated pores. Cephalic portion anterior to mouthparts comparatively large.

Notes.—This species resembles *A. longicauda* in shape and size but differs in that the cauda has longitudinal bands and striations and the precaudal bands and furrows are not present.

Type Reg. No. T5782 and paratype No. T5783 in the Queensland Museum.

Fig. 11.

Ancepaspis striata. Outline of Adult Female, X 110.

Fig. 12.

Ancepaspis striata. Enlargement of Pygidium, X 500.

Subfamily DIASPIDINAE.

Tribe Diaspidini.

Andaspis numerata new species.

(Figs. 13 and 14.)

Locality and host.—Queensland: Danbulla, on *Cedrela toona* Roxb. var. *australis* (F. Muell.) C.DC., Oct. 1938.

Habit.—Insects singly under felty fungus on twigs.

Recognition characters.—Adult female elongate-oval, membranous; length of slide specimen 1·2 mm., width 0·5 mm. Pygidium large, with two pairs of lobes. Median lobes one-and-a-half times as wide as long; inner margins subparallel, with a subapical indentation; outer margin angled, distal portion larger with four or five indentations, almost serrate; apex rounded. Second pair of lobes bilobed, the loblet small, triangular and almost independent. Third and fourth lobe positions marked by sclerotized serrations. Basal scleroses of median lobes elongate, longer than the lobes, one quarter as wide, arising medianly from the bases of the lobes. Two gland spines in each of the second, third, and fourth

Fig. 13.

Andaspis numerata. Outline of Adult Female, X 95.

Fig. 14.

Andaspis numerata. Enlargement of Pygidium, X 250.

interlobal spaces, simple, longer than the lobes. Macroducts with marginal orifices placed one in the first interlobal area, two in each of the second and third areas, one in the fourth; other dorsal ducts small and in segmental series. Perivulvar pores in five groups, 10 in the median, 17 to 19 in the anterior lateral groups, 8 to 10 in the posterior lateral groups. Anal opening anteriorly in pygidium. Vulva slightly less than half the distance of the anal opening from the lobes. Anterior spiracles with two or three associated pores.

Notes.—The basal scleroses may be slightly apically enlarged. Sometimes there may be three gland spines in the fourth interlobal space, and a small one in the first space.

This species has some resemblance to *A. hawaiiensis* (Maskell) but differs in having the second pair of lobes and no marginal spurs on the third and fourth abdominal segments.

Type Reg. No. T5784 and paratypes Nos. T5785 and T5786 in the Queensland Museum.

Aulacaspis australis new species.

(Figs. 15 and 16.)

Locality and host.—Queensland: Sandgate, on *Bruguiera gymnorhiza* Lam., Apr. 1947.

Habit.—Insects scattered on leaves. Scales subcircular, white, length 1·5 mm.; pellicles brownish green.

Fig. 15.

Aulacaspis australis. Outline of Adult Female, X 100.

Recognition characters.—Adult female elongate; length of slide specimen 1·1 mm.; width 0·65 mm.; prosoma large, as wide as and almost as long as the length of the postsoma. Prosoma tubercles present but not prominent. Pygidium large, not differentiated, apex broadly rounded. Median lobes moderately short, divergent; inner margins broadly rounded, basally close; outer margins and apex broadly rounded. Lobules of the second pair of lobes similar, the outer lobule slightly smaller than the inner lobule. Third pair of lobes similar to the second pair. Gland spines single in the first, second and third interlobal spaces. Eight macroducts with marginal orifices. Other ducts in segmental series on abdominal segments; first segment with 3 or 4 ducts in anterior group, 5 in posterior group; second segment with 5 in anterior group,

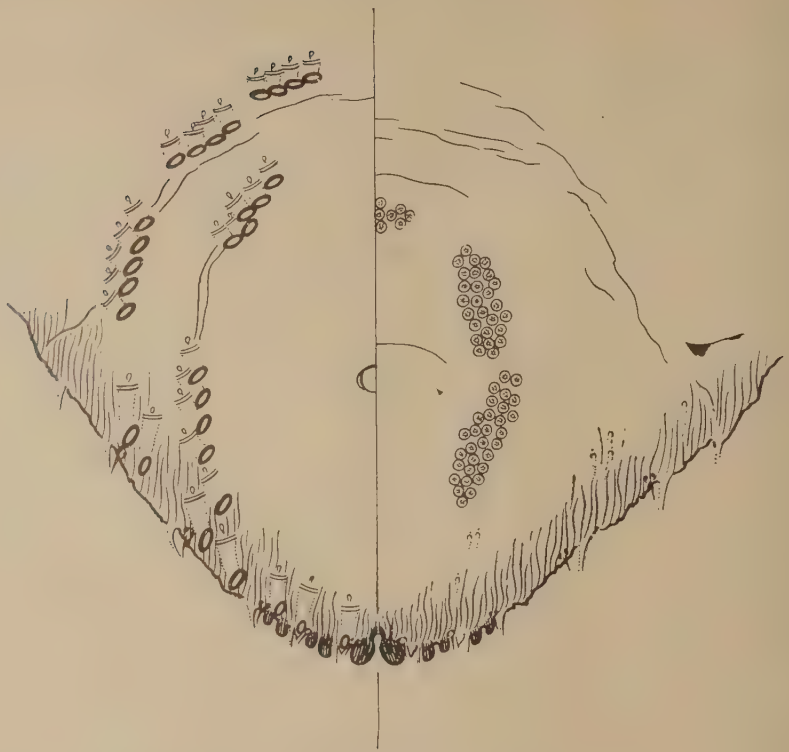

Fig. 16.

Aulacaspis australis. Enlargement of Pygidium, X 320.

posterior divided into a submedian group of 4 or 5 and submarginal group of 5 or 6; third segment with 4 or 5 in anterior group, 4 or 5 in the submedian and 6 or 7 in the submarginal; fourth segment with 4 in the anterior, 4 in the submedian and 5 in the submarginal; fifth segment, anterior group absent, 4 or 5 in the submedian, 4 or 5 in the submarginal; a row of ducts absent on the sixth segment. Small marginal ducts present on abdominal segments two and three. Perivulvar pores in five groups; 12 in anterior group, 26 to 28 in anterior lateral groups, 26 to 30 in posterior lateral groups. Anterior spiracles with 12 to 14 associated pores; posterior spiracles with 2 or 3 associated pores.

Notes.—In some specimens one of the posterior groups of ducts on the first abdominal segment may be absent.

This species has a close resemblance to *A. crawii* (Cockerell) but differs in having neither dorsal ducts on the sixth abdominal segment nor any indication of a fourth pair of pygidial lobes.

Type Reg. No. T5772 and paratypes Nos. T5773 to T5775 in the Queensland Museum.

Doriopus bilobus new species.

(Figs. 17 and 18.)

Type locality and host.—Queensland: Gayndah, on *Acacia bidwillii* Benth., Oct. 1954 (L. Pedley).

Habit.—Insects mostly single, embedded partly or wholly in the copious corky tissue on branches of the host. Adult female enclosed in the enlarged second stage nymph, which is olive green in colour, with a length to 1·0 mm. and width to 0·75 mm.

Fig. 17

Doriopus bilobus. Outline of Adult Female, X 130.

Recognition characters.—Adult female subcircular, membranous; length of slide specimen 0.75 mm., width 0.7 mm. Pygidium not differentiated, posterior part lightly sclerotized, with median pair of lobes only. Lobes large, one-and-a-half times as long as wide, contiguous; inner margins straight and parallel; outer margins constricted basally, broadly curved to the apex, with four latero-apical indentations. Basal scleroses and paraphyses absent. Gland spines shorter than the lobes, simple derm projections in a continuous

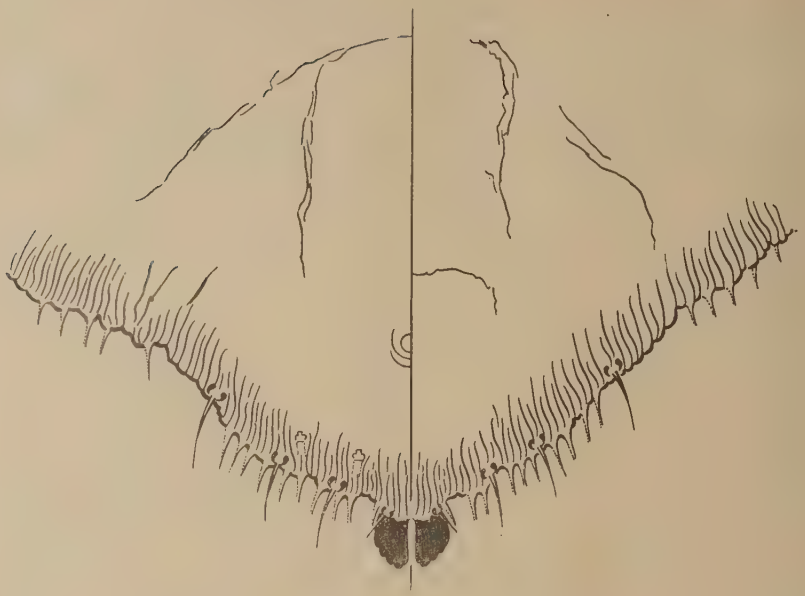

Fig. 18.
Doriopus bilobus. Enlargement of Pygidium, X 280.

series on the pygidium and extending anteriorly on abdominal segments, progressively smaller until as small dome-shaped processes. Spines adjacent to the lobes shorter than the lobes, other pygidial spines longer than the lobes. Dorsal ducts few and indistinct, short, orifices marginal, possibly one in each of the first and second interlobal areas. Anal opening round, moderately large in an apparent short anal tube, four times the length of the lobes from the bases of the lobes. Anterior spiracles with two or three associated pores. Posterior spiracles placed well forward.

Notes.—In some specimens the lobes may be twice as long as wide with the indentations on a somewhat blunt apex. The lobes also may be slightly constricted basally on the inner margin. There may be marginal incisions on each side of the pair of lobes, making the lobes appear as surmounting an apical pygidial projection.

Type Reg. No. T5791 and paratypes Nos. T5792 to T5794 in the Queensland Museum.

Phenacaspis difissata new species.

(Figs. 19 and 20.)

Locality and host.—Queensland: Tugun, on *Banksia integrifolia* L.f., Dec. 1948.

Habit.—Insects singly on under leaf surface. Scales elongate-oval to pyriform, white, length 1·5 mm.

Fig. 19.

Phenacaspis difissata. Outline of Adult Female, X 100.

Recognition characters.—Adult female elongate-oval, membranous; length of slide specimen 1·1 mm., width 0.8 mm. Pygidial margins almost straight, placed at or near a right angle; apex deeply incised or cleft, the cleft yoked basally. Median pair of lobes lining the cleft, barely exceeding the pygidial margin, bases separated, margin long, broadly curved and finely serrate. Second pair of lobes with lobules subtriangular; inner lobule margins entire, outer lobule with one or two indentations on outer margin; third pair of lobes with inner lobule triangular, outer lobule broad and short with serrations. Fourth lobe position marked by a chitinous marginal serration. Gland spines single on pygidium, in groups on prepygidial segments. Six

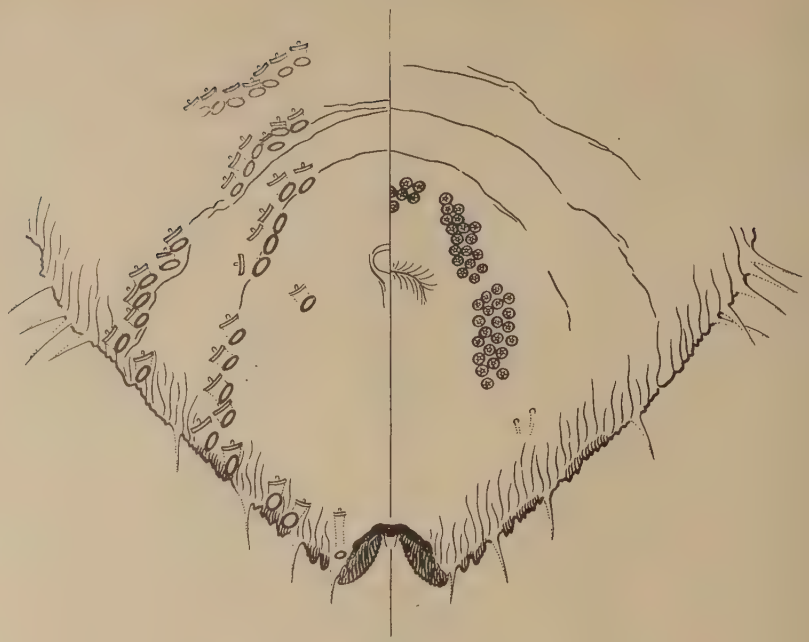

Fig. 20.

Phenacaspis difissata. Enlargement of Pygidium, X 230.

pygidial macroducts with marginal orifices. Other ducts in segmental series; first abdominal segment with 0 to 4 ducts in a submarginal group; second segment with 8 to 10 submarginal, 5 to 7 submedian; third segment with 7 or 8 submarginal, 7 submedian; fourth segment with 5 or 6 submarginal, 6 submedian; fifth segment with 3 or 4 submarginal and 4 or 5 submedian; sixth segment with 0 to 2 submedian. Groups of smaller ducts submarginally on abdominal segments one and two and on the metathorax. Perivulvar pores in five groups, 10 pores in the median group, 18 to 20 in the anterior lateral and 20 to 23 in the posterior lateral groups. Anterior spiracles with four or five associated pores. A group of three or four short-based spines both posterior to and anterior to the posterior spiracles. Anal opening round, placed anteriorly on the pygidium.

Notes.—This species resembles *P. megaloba* (Green) in having a deep pygidial incision but differs in having a segmental series of ducts on the second abdominal segment. The third pair of lobes may be sclerotized marginal serrations similar to those in the fourth lobe position; sometimes the serrations in the latter position also may be indistinct. The ventral groups of small, broad-based spines resemble those of *P. eugeniae* (Maskell) but *P. difissata* differs from that species in having two pairs of these groups of spines, a large pygidial incision and longer pygidial lobes.

Type Reg. No. T5789 and paratype No. T5790 in the Queensland Museum.

Poliaspis attenuata new species.

(Figs. 21 and 22.)

Locality and host.—Queensland: Yarraman, on *Croton insularis* Baill., Sept. 1948.

Fig. 21.

Poliaspis attenuata. Outline of Adult Female, X 95.

Habit.—Insects single, lining the margin of leaves. Scales linear, white in colour, 3·5 mm. in length; pellicles dark orange colour.

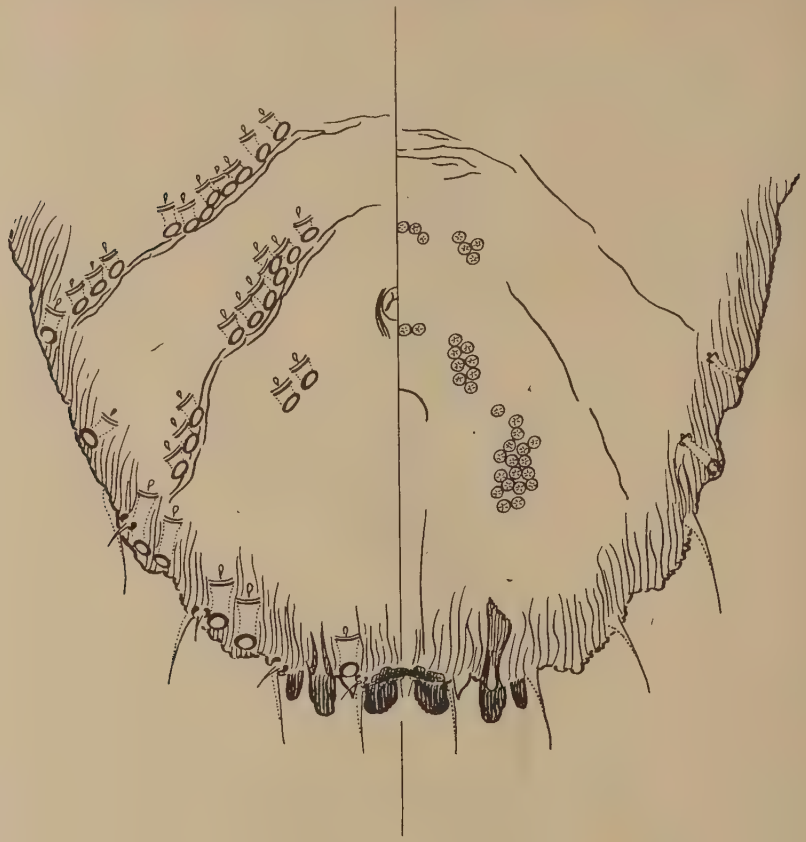

Fig. 22

Poliaspis attenuata. Enlargement of Pygidium, X 300.

Recognition characters.—Adult female very long and slender, membranous; length of slide specimen 1·6 mm., width 0·3 mm. Pygidium broadly rounded with two pairs of lobes. Median lobes longer than wide, asymmetrical, separated by a lobe's width, margin indented, almost serrate. Second pair of lobes with the inner lobule longer than wide projecting beyond the median lobes, outer lobule similar to but smaller than the inner. Gland spines single on pygidium. Spines small. Seven macroducts with marginal orifices. Other ducts in segmental series; third abdominal segment with three in the submarginal group and seven to nine in the submedian group; fourth segment with three submarginal, eight submedian; fifth segment with

four submarginal and five or six submedian; sixth segment with two submedian. Perivulvar pores in eight groups; ancilliary anterior group with six pores, ancillary lateral groups with four pores, the three groups placed closely together; normal anterior group with four pores, normal anterior lateral groups with eight or nine and posterior lateral groups with 18 or 19 pores. Anterior spiracles with two or three associated pores. Anterior and posterior spiracles widely separated. Ventral body surface with transverse fine fingerprint sclerotization.

Notes.—The very long body of this species and *P. elongata* separates them from all other species in the genus. This species differs from *P. elongata* in having the median lobes longer than wide and asymmetrical.

Type Reg. No. T5798 and paratype No. T5799 in the Queensland Museum.

Poliaspis elongata new species.
(Figs. 23 and 24).

Fig. 23.
Poliaspis elongata. Outline of Adult Female, X 65.

Locality and host.—Queensland: Tugun, on *Leptospermum whitei* Cheel, Sept. 1947.

Habit.—Insects single, lining the margin of leaves. Scales elongate, white, length 2·5 mm., pellicles dark orange in colour.

Fig. 24.

Poliaspis elongata. Enlargement of Pygidium, X 250.

Recognition characters.—Adult female long and slender, membranous; length of slide specimen 1·7 mm., width 0·4 mm. Pygidium rounded, with two pairs of lobes. Median lobes wider than long, each practically symmetrical, intervening cleft basally yoked, small. Second pair of lobes with lobules independent; inner lobule almost one-and-a-half times as long as wide, not projecting beyond the median lobes; outer lobule similar to the inner but much smaller. Gland spines single on pygidium. Spines small. Seven macroducts with marginal orifices. Other ducts in segmental series; third abdominal

segment five to seven in the submarginal group, five to 10 in the submedian group; fourth segment with five in the submarginal, six to eight in the submedian; fifth segment with three to five in the submarginal, five to seven in the submedian, sixth segment with two or three in the submedian group. Perivulvar pores in eight groups; ancilliary anterior group with six to eight pores, ancillary lateral groups with four to seven; normal anterior group with six to eight pores, normal anterior lateral groups with eight to 14, posterior lateral groups with 18 to 23. Anterior spiracles with two to four associated pores. Anterior and posterior spiracles widely separated.

Notes.—This species is close to *P. attenuata* but differs in having the medium lobes wider than long and each lobe practically of symmetrical shape.

Type Reg. No. T5795 and paratypes Nos. T5796 and T5797 in the Queensland Museum.

Locality and host.—Queensland: Kenmore, on *Callistemon viminalis* (Soland. ex Gaertn.) G. Don. ex Loud., Apr. 1958.

Fig. 25.

Morganella nudata. Outline of Adult Female, X 95.

Tribe Aspidiotini.

Morganella nudata new species.

(Figs. 25 and 26.)

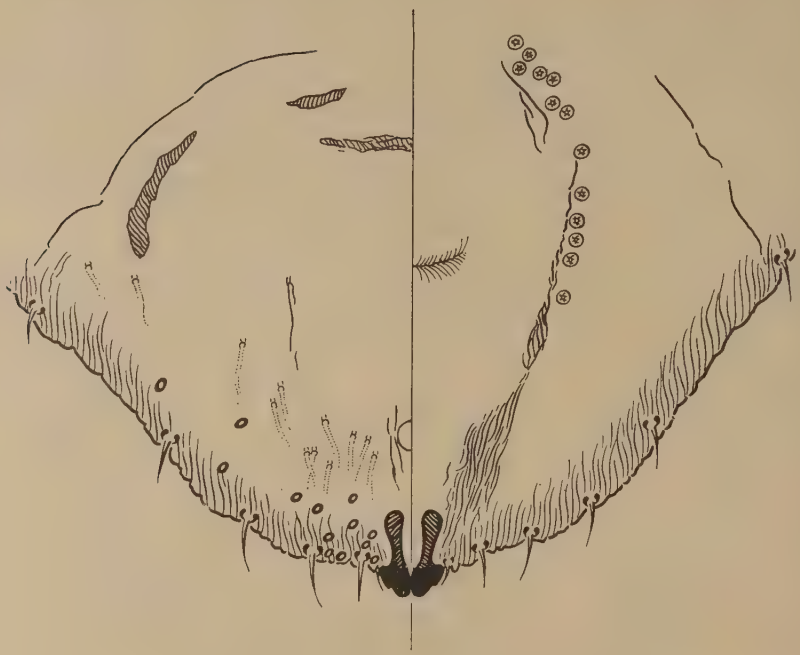

Fig. 26.

Morganella nudata. Enlargement of Pygidium, X 240.

Habit.—Insects single and sparse, under corky tissue on twigs.

Recognition characters.—Adult female broadly pyriform to subcircular, membranous; length of slide specimen 1·0 mm., width 0·8 mm. Pygidium large, not differentiated, apex broadly rounded, median pair of lobes only. Lobes longer than wide, convergent; inner margins adpressed for the distal half; outer margins convergent, notched midway; apex rounded. Basal scleroses large, divergent, basally one-half to two-thirds as wide as the lobes, one-and-a-half times as long as the lobes and apically enlarged. Paraphyses and plates absent. Spines adjacent to the lobes shorter than the lobes, other spines longer. Dorsal ducts sparse, slender, moderately long, reaching almost to the anal opening in the medial region; five ducts in the first furrow; six in the second furrow. Lateral and median dorsal scars present. Sclerotized arms from the pygidial apex and paravulvar folds co-linear. Perivulvar pores in four groups, six to eight in the anterior lateral groups and five to seven in the posterior lateral groups.

Notes.—The notch on the outer margin of the lobes may be almost subapical and sometimes two small indentations may be present.

This species has some resemblance to *M. longispina* (Morgan) but it differs in having perivulvar pores and no plates.

Type Reg. No. T5787 and paratype No. T5788 in the Queensland Museum.

ACKNOWLEDGEMENT.

Mr. William Manley, Departmental Illustrator, made all the drawings.

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FREEZE-DRYING OF STARTER CULTURES

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SUMMARY.

Cultures dried by the Rayner method retained their vitality for up to seven months, but in most cases a high cell mortality accompanied the drying.

Self-frozen cultures prepared with Gray's apparatus gave lower survival populations than externally frozen cultures. The mortality on storage is high in all drying procedures.

I. INTRODUCTION.

Many biological fluids, including suspensions of bacteria, contain heat-labile proteins and other substances which lose their biological activity when highly concentrated or subjected to temperatures in excess of 30 deg. C. Bacterial suspensions cannot therefore be preserved by drying from aqueous solutions.

As early as 1909 Shackell suggested that deterioration of labile material at high concentrations and elevated temperatures could be overcome if the solutions were dried from the frozen state and then placed over a desiccant in a high vacuum. Under these circumstances evaporation was sufficiently rapid for the solution to remain frozen. Drying then occurred by ice sublimation from the frozen state without an intermediate liquid phase. This process of freezing and drying provides the only reliable method of preserving labile materials such as bacteria. The method has been widely used both in the laboratory and in industry for the preservation of cheese starter organisms.

The purpose of the present investigation was to examine some of the methods available in a small laboratory for the freezing and drying of "starter" cultures and to examine some of the factors upon which the efficiency of freezing and drying depends.

II. GENERAL CONSIDERATIONS.

(1) Fundamental Requisites.

Flosdorf, Hull, and Mudd (1938) list the requisites of the technical set-up for freeze-drying as follows:—

(*a*) A device to ensure the maximum rate of evaporation from the material undergoing desiccation which is compatible with the total operation and with the safety of the product.

(*b*) A device which ensures the maximal rate of flow of water vapour from the evaporating surface to the surface where the vapour is removed.

(*c*) A device to achieve the maximum economy and efficiency of removal of water vapour whether by condensation or evaporation.

These requisites may be satisfied in a variety of ways and consequently considerable differences in technical design are in evidence in the methods employed for the freeze-drying of bacterial cultures.

(2) Freezing of Culture.

The first step in the freeze-drying process is the freezing of the culture to be dried. This may be achieved either by self-freezing or by the application of an external freezing mixture. When self-freezing is practised, preliminary degassing must be employed to avoid frothing. This may be accomplished by shutting off the vacuum pump for a minute or two as soon as the fluid in the ampoules commences to bubble. When external freezing is employed, a temperature below the eutectic must be used; otherwise the liquid phase which separates from the frozen remainder will not only froth violently at the subsequent evacuation stage but the culture will be liquid-dried rather than freeze-dried, with consequent loss of biological activity (Bradish 1947).

(3) Vacuum Sublimation of Ice.

In any sublimation process a solid substance is vaporized in such a way that the intermediate liquid phase is eliminated. The vapour which is removed is disposed of in some other part of the apparatus, while the non-subliming substances are retained in the ampoules. Vacuum sublimation is employed since the elimination of the liquid phase is a critical factor. The water vapour may be disposed of by being condensed at a low temperature, by the use of desiccating substances such as P_2O_5, H_2SO_4 and/or silica gel, or by direct pumping.

The efficiency with which vacuum sublimation can be carried out is a function of many physical variables.

(4) Design of Equipment.

In the range 10 to 1,000 microns, where drying by sublimation occurs, resistance to flow is inversely proportional to the fourth power of the radius and directly proportional to the length of the line. Thus a piece of apparatus should be so designed that there are few or no restrictions to the flow. This is not easy to accomplish in practice and some of the apparatus used in the experimental work described in this paper suffers in some respects from this deficiency.

III. FREEZE-DRYING OF "STARTER" CULTURES.

The freeze-drying of bacterial cultures is not merely of academic significance. It has found many applications in the fermentation industries,

including the dairying industry. Traditionally, "starters" used for the manufacture of cheese have been distributed to factories in chalk-litmus-milk. This method has proved very satisfactory and is widely used in many localities. But in recent years dairy laboratories have adopted the practice of preserving stock cultures in the dried state and of distributing cultures to cheese factories in the dried or powdered form. The Dairy Research Section of the C.S.I.R.O. commenced an Australia-wide distribution of cultures in this form in January, 1955.

IV. OUTLINE OF EXPERIMENTAL WORK.

(1) Using the Rayner Method.

Using the Rayner method the following investigations were made:—

(*a*) The effect of storage on vitality and morphology.

(*b*) The determination of survival populations after 5, 6 and 7 months' storage respectively.

(2) Using Gray's Method.

Using Gray's method the following investigations were made:—

(1) The determination of the effect of freezing and drying on survival populations using self-freezing.

(2) The determination of the effect of freezing and drying on survival populations using external freezing.

(3) The determination of strain susceptibility to the effects of freezing and drying.

(4) The effect of pre-centrifuging of cultures on survival populations.

V. EXPERIMENT USING RAYNER'S METHOD.

(1) Rayner's Method.

The cultures to be dried are grown overnight in sterile skim-milk. Drops of the culture are transferred by means of a capilliary pipette to sterile pieces of Cellophane (approx. $\frac{3}{4}$ in. square) resting in a sterile petri dish.

The dish and its contents are then transferred to a vacuum desiccator containing silica gel, the lid of the dish being left slightly open. The vacuum desiccator is then exhausted by means of a pump. The cultures freeze and dry within 20 minutes. The cultures are nevertheless left overnight in the exhausted desiccator. The Cellophane strips are then transferred to sterile ampoules; these are placed in the desiccator, which is again evacuated and left overnight. The ampoules are then evacuated and sealed at the constriction in a blow-pipe flame.

After sealing, each tube of dried culture was tested for vacuum (to ensure that there were no leaks) by means of a high frequency vacuum tester

connected to the A.C. mains supply. Any ampoules exhibiting crazing were discarded, as it was found that such ampoules did not hold their vacuum very well.

(2) Effect of Storage on Vitality and Morphology.

(*a*) *Preparation of cultures.*—The cultures used were:—"GB", "H.P.", "Q_4", "R_1", "S_1", "ML_2", "ML_3", "F_1", and "MHS_4". The cultures were inoculated into sterile skim-milk and incubated for 24 hr. at 22 deg. C. preparatory to being dried.

Vitality test:—The cultures were subjected to the following vitality test. Milk pasteurised at 145 deg. C. for 30 min. is dispensed in 150 ml. quantities and the temperature raised to 30 deg. C. Then 1 per cent. starter is added and the starting time of the test is calculated from this point. Thirty minutes later five drops of rennet are added and one hour later the curd is cut to $\frac{1}{4}$ in. cubes with a knife. The temperature is then raised to 37 deg. C., which is maintained for two hours. The whey is then drained from the curd, and incubation at 30 deg. C. for a further hour follows. Nine millilitres of whey are titrated with ·1N NaOH, using phenolphthalein as an indicator.

(*b*) *Microscopic examinations.*—These were conducted by inoculating tubes of sterile skim-milk with one loopful of culture, incubating at 22 deg. C. and 37 deg. C. respectively. After four hours' incubation smears are prepared, stained with Newman's stain and examined microscopically.

Vitality tests and microscopic examinations were conducted on cultures before and after storage for periods up to seven months. No fewer than eight cultures were dried in each trial. Results and observations are recorded in Table 1.

In cases where clotting did not occur, the ampoules had not held their vacuum or the cultures had very poor initial vitality. The vitality of the "starters" did not appear to be appreciably affected by drying and storage in the dried state for periods up to seven months.

With one or two exceptions, morphology did not appear to be affected by drying and storage.

(3) Determination of Survival Populations after Storage.

The determination of viable counts before and immediately after freeze-drying and after storage, though subject to certain inaccuracies, does afford some information concerning the effect of drying on the initial population. Viable counts before and after storage for five, six and seven months are tabulated in Table 2. Though all cultures exhibited a high mortality on drying and storage, clotting nevertheless occurred in all cases within 36 hr. The viable counts were made on Hunter's media (1946), which gave large, easily countable colonies of the "starter" organisms.

Table 1.

RELATIONSHIP BETWEEN STORAGE TIME, VITALITY AND MORPHOLOGY.

Storage Period (months).	Culture.	Vitality Test.		Morphology.			
				Before Freeze-drying.		After Freeze-drying.	
		Before Freeze-drying.	After Freeze-drying.	At 22°C.	At 37°C.	At 22°C.	At 37°C.
3	GB	26	27	Typical	Typical	Typical	Long chains
	HP	35	35	,,	,,	,,	Typical
	Q4	25	40	,,	,,	,,	,,
	R1	19	29	,,	,,	,,	,,
	S1	3	..	,,	,,	,,	,,
	ML2	19	20	,,	,,	,,	,,
	ML3	29	31	,,	,,	,,	Long chains
	MHS4	27	28	,,	,,	,,	Typical
4	GB	30	35	Typical	Typical	Typical	Typical
	HP	30	46	,,	,,	,,	,,
	Q4	32	24	,,	,,	,,	,,
	R1	21	30	,,	,,	,,	,,
	S1	3	3	,,	,,	,,	,,
	ML2	4	2	,,	,,	,,	,,
	ML3	14	15	,,	,,	,,	,,
	MHS4	44	..	,,	,,	,,	,,
5	GB	36	27	Typical	Typical	Typical	Typical
	HP	39	34	,,	,,	,,	,,
	Q4	8	..	,,	,,	,,	,,
	R1	33	20	,,	,,	,,	,,
	S1	7	3	,,	,,	,,	,,
	S2	24	..	,,	,,	,,	,,
	ML2	31	24	,,	,,	,,	,,
	ML3	33	39	,,	,,	,,	,,
	F1	12	8	,,	,,	,,	,,
6	GB	32	..	Typical	Typical	Not examined as cultures possessed no vitality	
	HP	36	..	,,	,,		
	Q4	42	..	,,	,,		
	R1	25	..	,,	,,		
	S1	12	..	,,	,,		
	S2	18	..	,,	,,		
	ML2	14	..	,,	,,		
	ML3	33	..	,,	,,		
7	GB	32	29	Typical	Typical	Typical	Typical
	HP	31	39	,,	,,	,,	,,
	Q4	4	40	,,	,,	,,	,,
	R1	30	37	,,	,,	,,	,,
	S1	1	15	,,	,,	,,	,,
	S2	14	4	,,	,,	,,	,,
	ML2	22	17	,,	,,	,,	,,
	ML3	39	39	,,	,,	,,	,,

Table 2.

SURVIVAL POPULATIONS AFTER 5, 6 AND 7 MONTHS.

Storage Period. (months).	Culture.	Bacterial Estimates.	
		Before Freeze-drying.	After Freeze-drying.
5	GB	89,000,000	80,000
	HP	36,500,000	10,000
	Q4	28,400,000	10,000
	R1	49,200,000	10,000
	S2	8,500,000	10,000
	S3	L.A.	10,000
	ML2	47,000,000	40,000
	ML3	52,000,000	70,000
	F1	24,000,000	10,000
6	GB	48,000,000	10,000
	HP	19,000,000	10,000
	Q4	17,200,000	10,000
	R1	30,700,000	10,000
	S1	4,200,000	10,000
	S2	19,300,000	10,000
	ML2	30,900,000	10,000
	ML3	44,800,000	10,000
7	GB	52,700,000	970,000
	HP	32,600,000	10,000
	Q4	18,800,000	10,000
	R1	27,400,000	10,000
	S1	18,300,000	70,000
	S2	20,900,000	40,000
	ML2	36,000,000	100,000
	ML3	41,000,000	530,000

(4) Conclusions.

(*a*) The Rayner method requires only simple equipment and does not involve complex techniques.

(*b*) Cultures dried by this method retained their vitality for periods up to seven months.

(*c*) Drying was accompanied by a high cell mortality in practically all cases.

VI. EXPERIMENTS USING GRAY'S METHOD.

(1) Gray's Method.

Gray's apparatus consists of a hollow metal cylinder 18 in. high and 4 in. in diameter. The cylinder is sealed at the base and is fitted with a detachable vacuum-tight lid, similar in construction to the type commonly fitted to the McIntosh and Fildes anaerobic jar.

Several rows of metal ports communicating with the hollow centre and sloping slightly downward from the horizontal are arranged peripherally round the cylinder. A short length of pressure-tubing fitted with a release-type screw clamp is attached to each part.

The core of the cylinder is fitted with a nickelled copper gauze basket charged with silica gel. The moisture-laden air is drawn from the drying cultures by a central manifold. This air passes directly through the silica-gel in the basket. The exhaust manifold is fitted by means of a sleeve valve at the base of the cylinder to the exhaust pump.

Ampoules containing the cultures to be dried are inserted into the rubber parts, using a smear of lubricant, and the clips are loosened. Should the cultures froth during evacuation the pump is shut off for a minute or two.

(2) Determination of the Effect of Freezing and Drying on Survival Populations using Self-freezing.

Cultures were prepared as in previous experiments. The cultures were not pre-frozen in an external freezing mixture prior to the pump being switched on. Viable counts were made on the cultures prior to freeze-drying and then immediately after drying. The results obtained are shown in Table 3.

Survival rates on the whole were poor and rather erratic. A possible explanation of this is that some of the cultures were dried from the liquid and not from the frozen state.

Table 3.

PERCENTAGE SURVIVAL (AFTER DRYING—NO PRE-FREEZING).

Trial No.	Culture.	Viable Count, per 0·1 ml.		Percentage Survival.
		Before Freeze-drying.	After Freeze-drying.	
1	GB	475,000,000	289,000,000	61
	HP	789,000,000	38,000,000	5
	Q4	263,000,000	81,000,000	31
	R1	791,000,000	230,000,000	29
2	GB	1,000,000,000	1,047,000,000	100
	HP	1,222,000,000	332,000,000	27
	Q4	890,000,000	114,000,000	13
	R1	780,000,000	421,000,000	54
3	GB	2,080,000	1,200,000,000	58
	HP	1,160,000	134,000,000	12
	Q4	1,060,000	148,000,000	14
	R1	10,000,000	1,000,000	10
4	GB	1,960,000	701,000,000	36
	HP	1,210,000,000	1,000,000	1
	Q4	720,000,000	135,000,000	19
	R1	1,410,000,000	284,000,000	20

(3) Determination of the Effect of Freezing and Drying on Survival Populations using External Freezing.

The results of this experiment are recorded in Table 4. It will be observed that the figures are of a higher order and more uniform than those recorded in Table 3. Proper external freezing seems to ensure that the drying takes place from the solid and not from the liquid state, thereby ensuring that the biological activity of the system is preserved to a greater extent. The freezing mixture consisted of alcohol and dry ice.

Table 4.

PERCENTAGE SURVIVAL AFTER DRYING (WITH PRE-FREEZING).

Trial No.	Culture.	Viable Count per 0·1 ml.		Percentage Survival.
		Before Freeze-drying.	After Freeze-drying.	
1	GB	2,190,000,000	784,000,000	36
	HP	1,130,000,000	350,000,000	31
	Q4	440,000,000	92,000,000	21
	R1	1,030,000,000	259,000,000	25
2	GB	2,150,000,000	738,000,000	34
	HP	400,000,000	140,000,000	35
	Q4	520,000,000	104,000,000	20
	Q1	990,000,000	380,000,000	38

(4) Determination of the Influence of Pre-freezing on the Survival Populations of two Strains.

Two cultures—"GB", a lactis strain, and "HP", a cremoris strain—were pre-frozen by external freezing and dried. Counts were made before drying and immediately after drying to determine if any strain susceptibility to drying existed. The techniques employed were as described previously. Results are tabulated in Table 5.

On the whole, survival rates were high. "GB" exhibited more favourable results than "HP". However, this may be related more to morphology than to strain sensitivity to the effects of freezing and drying.

(5) Drying of Centrifuged and Uncentrifuged Cultures.

The centrifuged culture was prepared as follows. On the first day the culture was sown in 10 ml. sterile skim-milk and incubated at 22 deg. C. for 24 hours. On the second day, 1 ml. of the clotted culture was sown in 5 ml. of Hunter's broth and incubated. On the fourth day the broth culture was well shaken, transferred to a 15 ml. sterile centrifuge tube, and centrifuged at 3,000 r.p.m. for 30 min.

Table 5.

SURVIVAL POPULATIONS OF "GB" AND "HP".

Trial No.	Culture.	Viable Count per 0·1 ml.		Percentage Survival.
		Before Freeze-drying.	After Freeze-drying.	
1	GB	420,000,000	205,000,000	49
		390,000,000	219,000,000	56
		460,000,000	247,000,000	54
		380,000,000	254,000,000	67
2	HP	120,000,000	35,000,000	31
		160,000,000	32,000,000	50
		90,000,000	42,000,000	47
		180,000,000	34,000,000	19
3	GB	330,000,000	193,000,000	58
		300,000,000	225,000,000	75
		310,000,000	194,000,000	63
		330,000,000	152,000,000	46
4	HP	100,000,000	46,000,000	46
		100,000,000	59,000,000	59
		120,000,000	25,000,000	20
		150,000,000	26,000,000	17
5	GB	340,000,000	180,000,000	50
		320,000,000	206,000,000	64
6	HP	150,000,000	86,000,000	57
		240,000,000	92,000,000	38

The supernatant liquid was then decanted and the sediment re-suspended in 2 ml. of sterile skim-milk. This suspension was then frozen and dried. A routine skim-milk culture was then frozen and dried simultaneously. Viable counts were made as in previous experiments. Results are recorded in Table 6.

No marked improvement in survival rates was obtained by centrifuging the bacterial cells first and then suspending in sterile skim-milk. In the trials performed the uncentrifuged cultures had higher survival rates.

(6) Effect of Storage on Survival Populations.

Four cultures—"GB", "HP", "Q4", and "R7"—were pre-frozen and dried using the Gray technique.

Viable counts were made prior to freezing and drying and after one and two months' storage respectively. Percentage survivals are included in Table 7.

After two months the survival percentage was less than 1 per cent.

Table 6.

COMPARISON OF SURVIVAL RATES OF CENTRIFUGED AND UNCENTRIFUGED CULTURES.

Trial.	Culture.	Viable Count per 0·1 ml.		Percentage Survival.
		Before Freeze-drying.	After Freeze-drying.	
1	GB	240,000,000 230,000,000	188,000,000 173,000,000	77
	GB (Centrifuged)	370,000,000 400,000,000	227,000,000 226,000,000	59
2	HP	120,000,000 140,000,000	51,000,000 60,000,000	43
	HP (Centrifuged)	100,000,000 30,000,000	12,000,000 1,000,000	12

Table 7.

PERCENTAGE SURVIVAL AFTER STORAGE.

Culture.	Percentage Survival.		
	Before Storage.	1 month's Storage.	2 months' Storage.
GB	36	2	< 1
HP	11	1	< 1
Q4	19	1	< 1
R1	20	3	< 1

(7) Conclusions.

(*a*) Gray's apparatus is simple and easy to handle.

(*b*) Self-frozen cultures did not give survival populations as high as externally frozen cultures.

(*c*) The mortality in all drying procedures appears to be high on storage.

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(Received for publication June 19, 1959.)

A BIOCHEMICAL STUDY OF 141 STRAINS OF ENTEROBACTERIACEAE ISOLATED FROM TISSUES AND FLUIDS OF ANIMAL ORIGIN

By ELIZABETH TURNER, B.Sc., Asst. Bacteriologist, Animal Research Institute, Division of Animal Industry.

SUMMARY.

The following tests were performed on 141 strains of Enterobacteriaceae isolated from a variety of animal tissue and fluid submitted for bacteriological examination: motility, acid production from glucose, mannitol, adonitol, dulcitol, inositol, lactose, salicin and sucrose, gas production from glucose, indole production, gelatin liquefaction, hydrogen sulphide production, methyl red and Voges-Proskauer test, urea hydrolysis, growth in potassium cyanide and Koser's citrate, and deamination of phenylalanine.

The strains were grouped as Salmonellae 15, Arizona 2, Escherichia coli **19, Alkalescens-Dispar 8,** Escherichia freundii **2, Bethesda-ballerup 13,** Klebsiella ozenae **3,** Klebsiella pneumoniae **19,** Klebsiella **sp. 12, Cloaca 27, Providence 2 and ungrouped 19.**

The importance for further studies and pathogenicity assessment on lesser known strains of Enterobacteriaceae is stressed.

I. INTRODUCTION.

The family Enterobacteriaceae contains genera in which are included bacteria ranging from those with no recognised pathogenicity for animals to those with marked pathogenicity. Many possessing little or no pathogenicity as primary pathogens are important as secondary invaders. However, there are also numerous bacteria in this family whose pathogenicity has not been adequately assessed and as the infections caused by them are not as common as those produced by Salmonellae and Shigellae little attention has been given to their isolation and identification. Examples are bacteria of the Providence and Bethesda-ballerupensis groups.

In veterinary medicine, Salmonellae are recognised as potential pathogens and most veterinary diagnostic laboratories have long since adopted techniques for the selective isolation of these bacteria from animal tissues and fluids which are usually grossly contaminated with bacteria of intestinal or environmental origin. Considerable attention has also been given to the pathogenicity of biochemical and antigenic types of *Escherichia coli*, especially in the pathogenesis of infectious scours in calves.

The publication in 1956 (Cowan 1956) of a scheme separating the members af the Enterobacteriaceae into groups on biochemical characteristics suggested that it may be possible to pay more attention to the lesser known pathogens using methods consistent with the speed and economy necessary

in a diagnostic laboratory. Cowan's scheme is based to a large extent on the results obtained in the study of strains of human origin. It was considered desirable therefore to determine whether the tests described would be of value when employed for the identification of organisms isolated from specimens of animal tissues and fluids submitted to this Institute, especially those belonging to the groups Providence, Klebsiella, Bethesda-ballerupensis and Arizona.

This note describes the application of a limited range of biochemical tests to 141 strains belonging to the family Enterobacteriaceae.

II. METHODS.

(1) Source of Strains.

Strains were isolated on blood, MacConkey and Salmonella-Shigella agar used during the routine examination of specimens submitted to this laboratory. Special attention was given to strains of Enterobacteriaceae in samples of milk from cases of bovine mastitis, to strains that preliminary tests suggested were Salmonellae but did not agglutinate Salmonella group B, C, D or E antisera, and to strains suspected to be species of Klebsiella.

The strains were considered to belong to the family if they were gram-negative, non-sporing rods, which grew readily in media containing only meat extract or peptone, and fermented glucose.

(2) Examination of Strains.

The tests employed were those considered to be adaptable to facilities in this laboratory, and to enable biochemical characterisation of strains in the manner described by Cowan (1956), viz:—

Motility.—This was determined by microscopic examination of 6-and 24-hour broth cultures by the dark field method of illumination. In a few instances the semisolid medium method (Edwards and Ewing 1955) was used to check results obtained by the microscopic method.

*Fermentation Test*s.—Acid production from glucose, mannitol, adonitol, dulcitol, inositol, lactose, salicin and sucrose was determined using peptone broth containing 1 per cent. of the test substance with Andrade's solution as indicator. Durham tubes were included to detect gas production in tubes containing glucose. Media were incubated up to 21 days before being discarded as negative.

Indole Production.—This was detected by the addition of Ehrlich's reagent after the organisms had grown in tryptone broth for 24 hours at 37 deg. C.

Gelatin Liquefaction.—Nutrient gelatin stabs were incubated at 37 deg. C. for 21 days then cooled to about 4 deg. C. before noting whether liquefaction had occurred.

Hydrogen sulphide (H_2S).—Lead acetate impregnated filter papers were used to detect hydrogen sulphide production from cultures grown on nutrient agar slopes at 37 deg. C. for seven days.

Methyl Red Test (MR).—Methyl red solution was added to buffered glucose phosphate broth cultures after incubation at 37 deg. C. for three days.

Voges Proskauer Test (VP).—This was done on 3-day cultures of buffered glucose phosphate broth using Barritt's (1936) method.

Urease.—Urea hydrolysis was detected by Christensen's agar method (Christensen 1946).

Potassium cyanide Growth Test (KCN).—Braun's KCN medium as put forward by Moeller and described by Edwards and Ewing (1955) was used. The medium was deep frozen at —25 deg. C. and thawed immediately before use.

Growth in Koser's Citrate Medium.—The medium used was that described in Standard Methods for the Examination of Water and Sewerage (American Public Health Association 1946).

Phenylalanine Test.—The method used was that described by Henricksen (1950).

III. RESULTS.

One hundred and sixty-three isolates were collected for examination, but 22 subsequently proved either not to belong to the Enterobacteriaceae or gave positive tests for urea hydrolysis and phenylalanine deamination and were considered to be strains of Proteus. Proteus strains were not further considered.

Table 1.

SOURCE OF STRAINS.

Biochemical Grouping.	Animal Species.							
	Fowls.	Ducks.	Cockatoos.	Pigs.	Horses.	Cattle.	Dogs.	Sheep.
Salmonella	6	..	..	2	..	6	..	..
S. pullorum	1	..	..	..	..	..	..	..
Arizona	1	..	..	..	..	1	..	..
Escherichia coli	3	3	..	7	2	4	..	..
A–D group	7	..	..	1	..	..	..	..
E. freundii	..	..	..	..	..	2	..	..
Bethesda-ballerup	5	..	..	5	1	2	..	..
K. ozaenae	2	..	..	..	..	1	..	..
K. pneumoniae	5	..	2	1	..	9	1	1
Cloaca	7	..	..	4	..	16	..	..
Providence	..	..	..	..	..	2	..	..
Klebsiella sp.	..	..	1	..	..	11	..	..
Ungrouped	4	1	..	9	..	4	1	..
Total	41	4	3	29	3	58	2	1

Table 1 gives details of the number studied in each group and the animal species from which they were isolated. The biochemical characteristics of the strains are given in Tables 2, 3 and 4

Details of strains in individual groups other than Proteus are considered in the order of groups given by Cowan (1956). No isolates were placed in the categories of *Salmonella typhi, Shigella sonnei,* other Shigellae, *Klebsiella rhinocleromatis* or Hafnia.

(*a*) *Salmonella.*

Strains 28, 29, 30, 37, 40, 59, 60, 61, 62, 123, 124, 130, 137, 138 were Salmonellae.

These fifteen strains were only part of those isolated at this laboratory during the period in which this work was done. In many instances they were included as they did not agglutinate any of the antisera to Salmonella groups B, C, D, or E, although they conformed to the pattern of Salmonella in the biochemical tests used routinely* at this Institute.

Fourteen strains gave the results listed by Cowan (1956), except that Strains 28, 29, 37, 40, 59, 60, 61, 62, 123, 127 did not produce hydrogen sulphide. Strains 130 and 138 produced only slight blackening of the lead acetate paper and Strain 124 produced this only after three days' incubation.

Strain 146 was *S. pullorum* with characteristics as given in Table 2.

(*b*) *Arizona.*

Strains 43 and 45 gave reactions as listed by Cowan (1956) for this group. Strain 45 liquefied gelatin only after seven days' incubation. Strain 43 was isolated from a bovine liver and strain 45 from a chicken lung. The clinical and post-mortem results in both cases did not indicate that these strains were the cause of sickness.

(*c*) *Escherichia coli.*

Strains 3, 27, 32, 42, 44, 46, 56, 57, 70, 71, 73, 74, 76, 103, 120, 128, 129, 132, 134 were considered to be *E. coli.* Included are three strains, Nos. 132, 133 and 134, isolated from the heart, bone marrow and liver respectively, of a duck. These strains were non-motile and encapsulated. Another non-motile strain, No. 120, was isolated from the uterus of a pig. Strain 71 fermented adonitol within 24 hours. Considerable variation occurred in the fermentation of dulcitol, salicin and sucrose, and the ability to produce hydrogen sulphide.

(*d*) *Alkalescens-Dispar Group.*

Strains 4, 5, 6, 7, 8, 9, 14 and 16 belonged to the alkalescens-dispar group. Strain 16 produced acid from both lactose and dulcitol and strain 14 produced acid from lactose. Strains 4, 5, 6, 7, 8, 9 and 14 were isolated from one batch of chickens submitted by one owner.

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* In routine tests, any gram negative bacillus producing acid and gas from glucose, fermenting mannitol and maltose, but not fermenting sucrose or lactose or producing indole is considered to be Salmonella and additional proof is sought by doing the slide agglutination test with Groups B, C, D, and E Salmonella antisera.

Table 2.

BIOCHEMICAL REACTIONS OF STRAINS PLACED IN COWAN'S GROUPS.

Test.	Salmonellae.	*S.pullorum.*	Arizona.	*E. coli.*	A–D Group.	*E. freundii.*	Bethesda-ballerup.	*K. ozaenae.*	*K. pneumoniae.*	Cloaca.	Providence.
	14	1	2	21	8	2	13	3	19	27	2
Motility	+*	−	+	17+4−	−	+	+	−	−	+	−
Gas from glucose	+	+	+	+	−	+	+	+	+	+	−
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+
Acid from mannitol	+	+	+	+	+	+	+	+	+	+	−
Acid from adonitol	−	−	−	1+20−	−	−	−	−	+	10+17−	−
Acid from dulcitol	+	−	−	14+7−	1+7−	−	6+7−	−	8+11−	4+23−	−
Acid from inositol	6+8−	−	−	−	−	+	−	+	+	7+20−	+
Acid from lactose	−	−	−	+	2+6−	+	10+3−	+	+	25+2−	−
Acid from salicin	−	−	−	10+5−6NT	−	+	6+3−4NT	2+1NT	7+12NT	NT	+
Acid from sucrose	−	−	−	9+12−	−	+	10+3−	2+1NT	+	26+1−	+
Indole	−	−	−	+	+	−	−	−	−	−	+
Gelatin liquefaction	−	−	1+1−	−	−	−	−	−	−	+	−
H_2S production	4+10−	−	+	5+16−	−	+	12+1−	2+1−	−	13+14−	−
Voges-Proskauer test	−	−	−	−	−	−	−	−	+	+	−
Methyl red test	+	−	+	+	−	+	+	+	−	−	+
Urea hydrolysis	−	−	−	−	−	+	5+8−	+	+	+	−
Growth in KCN	−	−	−	−	−	+	+	+	+	+	+
Growth in Koser's citrate	+	−	+	−	−	+	+	+	+	+	+
Phenylalanine test	−	−	−	−	−	−	−	−	−	−	+

* Unless otherwise stated, all strains in the category showed the result indicated.
NT = Not tested.

(*e*) *Escherichia freundii.*

Strains 101 and 102 were both isolated from bovine milk samples and had the biochemical characteristics of *E. freundii*. Two days' incubation was required for the production of hydrogen sulphide and the hydrolysis of urea. Acid production in inositol was slight.

(*f*) *Bethesda-ballerup.*

Strains 2, 17, 20, 58, 55, 53, 85, 86, 88, 127, 142, 143 and 145 were considered to belong in the Bethesda-ballerup group. These strains gave variable reactions in dulcitol, lactose, salicin, sucrose, hydrogen sulphide and urease.

Acid production from dulcitol occurred after 24 hours' incubation with strains 86, 142, 143 and 145, and 48 hours in strains 53 and 55.

No strain fermented lactose within 24 hours. Strain 58 did so after two days' incubation, strains 53 and 55 after three days, strains 142 and 143 after five days, strains 2, 17, 28 and 86 after seven days and strain 145 after 12 days.

Strains 85, 86, 88 and 127 were not tested for salicin fermentation. Positive results for salicin were obtained within 24 hours in strains 2, 17, 20, 53 and 58, and 48 hours in strain 55. Acid production in sucrose was delayed in strains 85, 88, 142, and 145. The longest period noted was 16 days for strain 142.

Hydrogen sulphide production was slight with strains 85, 88, and 127, and delayed for two and five days with two strains, 142 and 145.

(*g*) *Klebsiella ozaenae*

Strains 1, 33 and 105 had the biochemical characteristics of *K. ozaenae*. Strains 1 and 33 were isolated from chickens and strain 105 from a bovine milk sample. Strain 33 was isolated from liver and lung from a week-old chicken. Strain 1 was isolated from the eye of a 6-week-old Australorp chicken. Eight birds out of 200 seen had conjunctivitis with swollen eyelids. On autopsy, the bird submitted had a sero-tracheitis and *Klebsiella ozaenae* and *Lactobacillus* sp. were isolated from the eye discharge and alpha haemolytic streptococci from the trachea.

(*h*) *Klebsiella pneumoniae*

Strains 13, 91, 92, 94, 95, 98, 99, 104, 116, 135, 147, 149, 150, 151, 157, 158, 159, 162 and 163 had biochemical characteristics similar to those described by Cowan (1956) for *Klebsiella pneumoniae*.

Ludford and Stevens (1958) have described the isolation of Strain 13.

Strains 91, 92, 94, 95, 98, 99, 104, 116 and 117 were isolated from bovine milk samples obtained from clinical cases of mastitis. The ovine strain (No. 135) was isolated from a mesenteric lymph node from which *Salmonella*

typhimurium was also isolated. The clinical history of the affected animal included enteritis with profuse diarrhoea.

Urea hydrolysis was sometimes delayed. Strains 13, 91, 92, 94, 95, 98, 99, 104, 116, 135, 157 and 162 were positive after 24 hours' incubation but the remaining seven strains gave only a weak positive reaction after 24 hours, requiring 48 hours' incubation to give the strongly positive result.

Table 3.

BIOCHEMICAL REACTIONS OF KLEBSIELLA STRAINS WHICH COULD NOT BE GIVEN SPECIES CLASSIFICATION.

Test.	Strain No.										
	107.	108.	109.	110.	111.	112.	113.	114.	115.	125.	161.
Motility	−	−	−	−	−	−	−	−	−	−	−
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+
Acid from mannitol	+	+	+	+	+	+	+	+	+	+	+
Acid from adonitol	−	−	−	−	−		−	−		+	+
Acid from dulcitol	+	+	+	−	+	+	+	+	−	−	+
Acid from inositol	+		−	−	+	+	−		+	+	+
Acid from lactose	+	+	+	+	+	+	+	+	+	+	+
Acid from salicin	+	+	+	+	+	+	+	+	+	+	+
Acid from sucrose	+	+	+	+	+	+	+	+	+	+	+
Indole	+	−	−	−	−	−	−	−	−	+	+
Gelatin liquefaction	−						−	−	−	+	−
H_2S production	−	−	−	−	−	−	−	−	−	−	−
Voges–Proskauer test	+	+	+	+	+	+	+	+	+	−	+
Methyl red test	−	−	−	−	−	−	−	−	−	+	+
Urea hydrolysis	+	+	+	+	+	+	+	+	+	+	+
Growth in KCN	+	+	+	+	+	+	+	+	+	+	+
Growth in Koser's citrate	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine test	−	−	−	−	−	−	−	−	−	−	−

(*i*) *Klebsiella* spp.

Table 3 gives the biochemical reactions of the 12 strains of Klebsiella-type strains which could not be placed in any of the three groups of Klebsiella. Strain 161 was isolated from the heart blood of a cockatoo, and all the others were isolated from bovine milk samples. Strains 107-114 resembled *K. pneumoniae* in all reactions except that they failed to ferment adonitol. Strains 107 and 111 produced acid from inositol after 10 days' incubation and strain 113 after 14 days incubation. Strain 115 did not ferment dulcitol but otherwise it also resembled *K. pneumoniae*.

(*j*) *Cloaca*

Strains 10, 11, 12, 21, 22, 23, 24, 25, 26, 31, 35, 36, 38, 39, 51, 67, 81, 93, 96, 97, 117, 131, 139, 140, 141, 148 and 156 could be placed in the group Cloaca.

Table 4.

Biochemical Reaction of 19 Strains which would not fit into the General Scheme of Classification.

Test.	Strain No.																	
	15.	18.	19.	34.	52.	69.	75.	78.	79.	82.	83.	84.	126.	160.	144.	47.	122.	64.	136.
Motility	−	+	−	+	+	+	+	+	−	+	+	−	+	−	+	+	−	−	+
Gas from glucose	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Acid from adonitol	−	+	+	+	+	−	+	−	+	−	+	−	+	−	−	−	−	−	+
Acid from dulcitol	−	+	+	+	+	−	+	−	+	+	−	+	$+^{5}$	+	−	−	−	−	+
Acid from inositol	+	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	$-^{2}$	+
Acid from lactose	−	*2	$+^{3}$	−	$+^{5}$	−	$+^{8}$	+	+	$+^{7}$	+	+	$+^{9}$	$+^{6}$	−	−	−	−	$+^{12}$
Acid from salicin	+	+	+	$+^{3}$	+	−	$+^{2}$	$+^{3}$	+	−	$+^{2}$	$+^{3}$	+	$+^{3}$	−	−	−	+	
Acid from sucrose	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−
Indole	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−
Gelatin liquefaction	−	$+^{15}$	$+^{20}$	−	−	−	−	−	−	−	−	−	$+^{20}$	−	−	−	$+^{2}$	+	−
H_2S production	−	−	−	+	+	+	+	−	−	−	+	+	−	−	−	−	+	−	+
Voges-Proskauer test	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Methyl red test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Urease hydrolysis	$+^{5}$	+	$+^{4}$	−	$+^{18}$	+	−	$+^{2}$	+	−	$+^{3}$	−	−	−	−	−	$+^{6}$	−	$+^{1}$
Growth in KCN	+	+	−	−	−	+	+	+	+	−	+	−	−	+	−	−	+	−	−
Growth in Koser's citrate	+	−	−	+	−	+	−	+	+	−	−	−	$+^{5}$	−	−	+	−	−	+
Phenylalanine test	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* Number of days is specified when positive result was recorded after first day's incubation in those tests observed daily.

Sixteen of the 27 strains were isolated from cattle.

The strains fermenting adonitol, dulcitol and sucrose all did so within 24 hours. Strains 21, 31, 117, 24, 93, 139, 81, 141 required two to five days to ferment lactose and strains 96, 97, 24 and 141 fermented inositol weakly only after two to seven days. Gelatin liquefaction occurred only after periods of six to 30 days. Strain 131 hydrolysed urea in 24 hours and the remaining 26 strains which gave a weak reaction in 24 hours were positive after 48 hours' incubation.

(*k*) *Providencia.*

Strains 65 and 66 isolated were classified as Providencia, although motility could not be demonstrated in either case. They were isolated from the lung and pleural fluid of a cow. Both gave a positive result for the deamination of phenylalanine but did not hydrolyse urea.

(*l*) *Ungrouped Strains*

Strains 15, 18, 19, 34, 52, 69, 75, 78, 79, 82 83, 84, 126, 160, 144, 47, 122, 64 and 136 could not be placed with confidence into any of the groups. Their biochemical reactions are given in Table 4.

IV. DISCUSSION.

Most specimens submitted for bacteriological examination to this veterinary diagnostic laboratory suffer a considerable degree of contamination either from post-mortem spread of bacteria within the carcase or by contamination when exposed to the environment at post-mortem. A large number of such contaminants are Enterobacteriaceae. The problem is to decide which isolates other than Salmonellae should be studied in more detail to permit recognition of possible pathogens.

Among the strains described above, there are some other than Salmonellae which could be pathogens.

Most Salmonellae isolated in this laboratory conform to the biochemical pattern given by Cowan (1956), but the scheme suggested by that author does not satisfy requirements for *Salmonella pullorum*. The potassium cyanide test is of considerable value in distinguishing Salmonellae from organisms of the Bethesda-ballerup group.

Arizona strains which may be potential pathogens may also be confused with Salmonellae, but in contrast with most Salmonellae they do not ferment dulcitol. Kauffman and Moller (1956) have described a method for separating Salmonella, Arizona and Bethesda strains based on growth in KCN and the production of amino acid carboxylases.

Escherichia coli and the Alkalescens-Dispar group have similar IMVIC reactions, but the latter are differentiated from *E. coli* by lack of motility, not fermenting lactose and failure to form gas from glucose. Three strains

isolated from a duck resembled *K. pneumoniae* in colonial appearance, but biochemically had characteristics of *E. coli*. Several strains (18, 52, 75, 82, 83, 84, 122) had IMVIC reactions of *E. coli* but could not be classified as such because of the number of atypical results given in the other tests.

The Bethesda-ballerup group may be confused with Salmonellae. Pathogenicity has been ascribed to them by several workers. Their colony types are identical with those of the Salmonellae, and as mentioned above, except for the KCN test the biochemical tests of the two groups may be similar. Some strains differ from Salmonellae in their ability to ferment lactose, dulcitol, salicin and sucrose and to hydrolyse urea. *Escherichia freundii* is clearly related to the Bethesda group but ferments lactose within 24 hours.

Pathogenicity has not been ascribed to *Klebsiella ozaenae*. The strains isolated were VP negative and MR positive whereas *K. pneumoniae* is VP positive and MR negative.

Klebsiella pneumoniae is a potential pathogen and it has been isolated from a number of bovine milk samples submitted to this laboratory.

Eleven of the Klebsiella strains could not be placed in any species. From the aspect of pathogenicity it is important that this group be studied so that potential pathogens may be recognised.

Cloaca sp. have colonial morphology similar to Salmonellae on blood agar, and on MacConkey's agar and Salmonella-Shigella agar if they are non- or late-lactose fermenters. Cloacae most closely resemble the *K. pneumoniae* group in their reactions, distinguishing features being that the former are motile, non-capsulated and liquefy gelatin, whereas *K. pneumoniae* is non-motile, frequently capsulated and does not liquify gelatin. All strains of Cloaca examined hydrolysed urea, but except in one case, hydrolysis was slower than that of *K. pneumoniae* strains. The majority of Cloaca strains did not ferment inositol or adonitol whereas acid was produced from these sugars by all *K. pneumoniae* strains. The MR-VP tests separate Cloaca and *K. pneumoniae* from the other Enterobacteriaceae. No pathogenicity has been ascribed to Cloaca strains.

Providencia strains have been associated with urinary tract infections and diarrhoeic diseases in man (Edwards and Ewing 1955). Their colonial morphology is similar to Salmonellae but biochemically they are unique in that they do not produce urease but do deaminate phenylalanine.

Nineteen strains could not be placed in any of the groups and require further detailed investigation using both biochemical and serological methods to determine their positions in the Enterobacteriaceae.

Even with the limited biochemical tests done in this study, it is apparent that there would be a considerable delay in reporting results if they were to be routinely done. In most cases, the examination requested is for Salmonellae and these can be usually isolated and identified within a few days.

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SOME ASPECTS OF OESTRUS IN CATTLE, WITH REFERENCE TO FERTILITY ON ARTIFICIAL INSEMINATION

2. CRYSTALLISATION PATTERNS IN CERVICO-VAGINAL MUCUS

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SUMMARY.

A study of crystallisation patterns in the cervical mucus of five cows is reported. The cows had calved 5–70 days before the trial period. Throughout the 46 days' experimental period 224 observations were made.

Evidence of strong oestrogenic activity was obtained in 32.5 per cent. of observations made within five days before or after an observed oestrus. Only 14.0 per cent. of observations were indicative of pronounced oestrogenic activity in samples taken within 5–10 days before or after an observed heat.

In samples which could not readily be related to an observed oestrus, some 47.8 per cent. of the patterns reflected pronounced oestrogenic influence.

The significance of these results in relation to the detection of oestrus in cattle and in the study of atypical reproductive states is discussed.

I. INTRODUCTION.

While oestrus is readily recognised in the dairy cow under normal conditions, its partial suppression under unfavourable conditions may render its detection by conventional methods difficult or impossible. Such conditions seem to obtain in certain of the beef cattle breeding areas of Queensland In addition, there is evidence that the syndrome referred to as "enzootic anoestrus" is widespread in some dairying regions of the State (McTackett 1956). It is important in such cases to determine if oestrus is completely suppressed and if ovulation occurs.

Furthermore, the efficiency of vasectomised "teaser" bulls, with or without raddling, in the detection of oestrus in beef cows is suspect. The importance of improving current methods of detecting oestrus is immediately apparent, particularly in respect of the application of artificial insemination to beef cattle.

Fluctuations in the characteristics of cervico-vaginal mucus have been utilized in physiological studies in women (Seguy and Simonnet 1933; Lamar, Shettles and Delfs 1940; Papanicolaou 1945; Pommerenke 1946; Abarbanel 1946) to indicate oestrogen activity, ovulation and early pregnancy (Roland 1952). Of particular interest have been the variations in the pattern of crystallisation of the mucus. Garm and Skjerven (1952) noted the incidence of fern-like patterns in the dried mucus is related to the level of systemic oestrogens. On the other hand, Bone (1954) considered that the incidence of fern-like patterns is inversely related to the level of circulating progesterone. It has been reported (Rydberg 1948) that the crystallisation is due to the presence of chloride ions in the mucus.

More recently, Alliston, Patterson and Ulberg (1958) made a study of the crystallisation patterns of cervical mucus in relation to oestrus in beef cattle. They noted that the fern-like patterns of crystallisation become evident about 3·5 days before oestrus.

In view of the favourable reports of the sensitivity of the crystallisation pattern of cervical mucus to fluctuations in the oestrogen-progesterone complex, the phenomenon was utilized in an attempt to detect fluctuations in systemic oestrogens in cows disposed to anoestrus.

II. MATERIALS AND METHODS.

In a study of oestrous behaviour of cows in the Nambour region of south-eastern Queensland (Fallon 1958), it was noted that the majority of cows exhibited oestrus within 55 days after calving. Six cows which had histories of longer intervals to first heat in previous seasons were selected for the trial. The animals had calved normally within 10 weeks before the experimental period.

The techniques used in collecting, treating and evaluating the mucus samples were similar to those described by Alliston, Patterson and Ulberg (1958). The sampling procedure is similar to the rectal fixation technique of artificial insemination and was performed by competent inseminator-technicians.

It became evident as the trial proceeded that the susceptibility of the genitalia to infection during periods of luteal dominance (Casida 1952; Rowson 1952) may present serious problems in this work. It was necessary to administer medication to three of the animals on the 5th day of the trial and to six on the 16th day. There was no evidence that the therapeutic measures, consisting of intra-uterine infusion of 1 g. streptomycin sulphate plus 50 ml. sodium sulphadimidine in each case, affected the mucus characteristics of the treated cows. On the 19th day of the trial one animal was deleted because of its susceptibility to infection.

The samples were collected at approximately 8.00 a.m. on each day during the 46 days' period from Aug. 12 to Sept. 26, 1958. Scoring of the

crystallisation patterns was performed independently by three observers on each day of sampling. The scores finally allotted were determined by discussion between observers.

A classification system similar to that of Alliston, Patterson and Ulberg (1958) was used. Scores were allotted on the following basis:—(1) no pattern; (2) patterns around bubbles only; (3) typical patterns covering less than half of the total smear area; (4) typical patterns covering more than half of the total smear area, but not complete; (5) typical patterns over complete area of smear, both short and long ferns being present; (6) typical patterns over complete area, only long ferns being present.

Oestrus records were based on observations by the co-operating farmer, who had many years' experience in dairy farming and an intimate knowledge of the behaviour characteristics of his herd.

Results are presented in relation to the five animals which were studied throughout the trial period.

III. RESULTS.

Particulars concerning calving and oestrus dates are set out in Table 1 It will be noted that one animal (E) exhibited oestrus five days before the trial commenced. Another (B) failed to display clinical symptoms of oestrus within at least 67 days after calving. It is unfortunate in some respects that anoestrus was not more marked in the experimental group. However, such difficulties are by no means uncommon in trials which must be based on predicted behaviour. The atypical oestrous cycles recorded for cow D are not unusual in their occurrence (Fallon 1958).

Table 1.

Breeding Histories of Trial Animals.

Cow.	Calving Date.	Dates of Oestrus*.
A	Aug. 7	Sept. 10, Sept. 28
B	Aug. 4	
C	July 30	Sept. 13, Oct. 4
D	July 26	Aug. 21, Aug. 31, Sept. 20, Oct. 2
E	June 3	Aug. 7, (Aug. 31–Sept. 1)†, Sept. 19, Oct. 1

* Oestrus data complete to 10–10–58.

† Oestrus was recorded on 2 succeeding days.

A total of 224 observations on cervical mucus was made. The distribution of the observations throughout the various ratings is presented in Table 2. It will be noted that scores of 5 and 6, which may be regarded as indicating a very strong tendency towards the display of fern-like patterns, were recorded in 78 (representing 34·8 per cent.) of the observations.

Table 2.

Distribution of Cervical Mucus Crystallisation Scores.

Score.	Animal.					Total.
	A.	B.	C.	D.	E.	
6	1	2	2	2	4	11
5	8	24	14	10	11	67
4	15	10	13	12	15	65
3	11	4	4	9	8	36
2	3	2	4	2	1	12
1	7	3	8	9	6	33
Total	45	45	45	44	45	224

When the data are considered more specifically in relation to the incidence of oestrus (see Table 3) it is noted that 26 out of 80 observations (32·5 per cent.) made within five days before or after an observed heat were classified as 5 or 6. Only 7 out of 50 observations (14 per cent.) made within 5–10 days before or after an observed heat were rated as 5 or 6. These results are in general agreement with those reported by other workers and indicate the importance of the follicular phase of the oestrous cycle in the manifestation of the phenomenon concerned.

Table 3.

Influence of Stage of Oestrus on Cervical Mucus Rating.

Score.	Days Before and After Oestrus.			
	± 0–5.	± 5–10.	± 10–.	
6	7	..	4	11
5	19	7	41	67
4	25	25	15	65
3	15	7	14	36
2	3	4	5	12
1	11	7	15	33
Total	80	50	94	224

However, observations made during phases apparently less influenced by follicular development are particularly interesting. Some 47·8 per cent. of observations (45 out of 94) made more than 10 days before or after an observed heat were recorded as 5 or 6. Indeed, in the case of cow B, which was noted above to have exhibited anoestrus for at least 67 days after calving, 57·8 per cent of mucus samples collected during the anoestrous phase reflected strong oestrogenic activity.

Attempts at the detection of cyclic tendencies in the observations which were not related to observed oestrous cycles were without avail, although, as indicated above, strong oestrogenic activity is suggested in the bulked data.

As noted previously, short oestrous cycles are by no means uncommon and satisfactory fertility may be expected after such cycles (Fallon 1958), indicating that ovulation occurs in most, if not all, cases. Thus, it has been of interest to consider mucus ratings in relation to such short cycles. The relevant data are presented in Table 4. A marked difference in mucus ratings is evident between typical and atypical oestrous cycles.

Table 4.

CERVICAL MUCUS RATINGS IN RELATION TO THE OCCURRENCE OF OESTRUS ASSOCIATED WITH ATYPICAL CYCLE LENGTHS.

Days Before Oestrus.	Average Mucus Score.		Days After Oestrus.	Average Mucus Score.	
	Typical Cycles (n = 7).	Atypical Cycles (n = 3).		Typical Cycles (n = 7).	Atypical Cycles (n = 3).
10 ..	3·3	4·3	0* ..	5·8	3·5
9 ..	3·4	5·0	1 ..	4·8	2·5
8 ..	3·4	4·7	2 ..	3·6	3·0
7 ..	3·5	3·7	3 ..	2·6	3·5
6 ..	2·6	4·3	4 ..	3·2	2·5
5 ..	2·7	4·7	5 ..	4·0	4·0
4 ..	4·0	2·5	6 ..	3·5	1·5
3 ..	4·6	2·5	7 ..	3·8	..
2 ..	4·3	1·0	8 ..	2·5	4·0
1 ..	4·8	2·0	9 ..	3·25	3·0
0* ..	5·8	3·5	10 ..	3·5	3·0

* Day of oestrus.

Indeed, in the case of cow D (see Fig. 1), were a base heat taken at Aug 21 and, in the method of Alliston, Patterson and Ulberg (1958), observations taken from the 16th day thereafter, a more typical rise in rating occurs

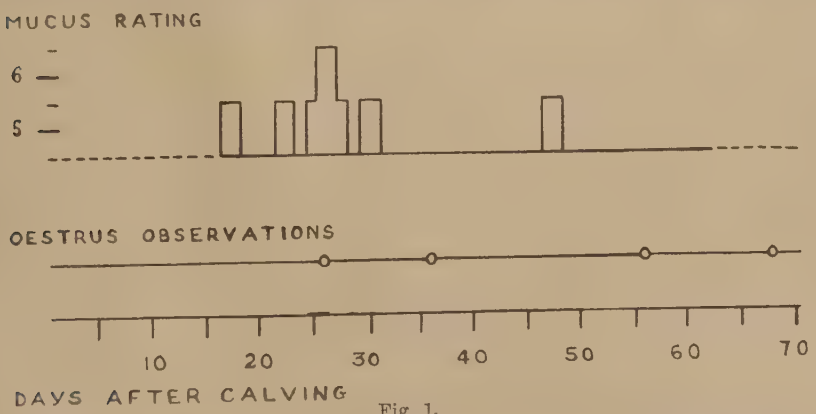

Fig. 1.

Cervical Mucus Crystallisation Patterns in Relation to Atypical Oestrous Cycles in Cow D.

at the 21st day, although no clinical symptoms of oestrus were noted. Furthermore, 9 days after the typical rise in mucus rating, to which reference has been made, a further heat with atypical mucus characteristics was recorded; it was followed in 12 days by an oestrus, for which no mucus ratings are available.

IV. DISCUSSION.

It is apparent that cervical mucus characteristics may be utilized as a valuable adjunct to current methods of detecting oestrus, particularly in relation to beef cattle breeding programmes. Certainly, if artificial insemination is being practised, sampling for cervical mucus beforehand would involve little additional time and labour for the competent operator. In the cow in which ovulation is imminent, the fern-like patterns in the cervical mucus are pronounced and readily observed.

There is ample evidence indicating that the changes in mucus characteristics during the follicular phase are essential for sperm penetration and viability in the mucus (Watson 1939; Campos da Pas 1951). However, a statistical relationship between mucus type and fertility remains to be established. There is ample evidence that tolerable levels of fertility may be obtained in the presence of cervical mucus markedly at variance in macroscopic appearance with "normal" oestrual mucus. This aspect is of further interest in relation to artificial insemination services, where inseminations are frequently performed 24 hours or more after the observation of oestrus and at a time when the peak mucus rating has passed.

The demonstration of high fern pattern ratings, indicating strong oestrogenic activity, during periods of anoestrus is of much interest. It serves, of course, to add further complication to the endocrinology of the syndrome. The complexity is accentuated when observations relating to short oestrous cycles are considered. It is apparent that the susceptibility of the cow's central nervous system to oestrogens (Asdell 1946) is variable and there is evidence that it may be completely suppressed under conditions of stress (Schaetz 1956; Fallon, unpublished data).

It is apparent that, in addition to their use in detecting specific changes in the reproductive state, crystallisation processes and other characteristics of cervical mucus (Blair and Glover 1956) may serve as useful aids in the elucidation of the more obscure aspects of bovine reproduction. The desirability of correlating the techniques more directly with hormone levels is obvious.

V. ACKNOWLEDGEMENTS.

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SOME ASPECTS OF OESTRUS IN CATTLE, WITH REFERENCE TO FERTILITY ON ARTIFICIAL INSEMINATION

3. BODY TEMPERATURE AND THE OESTROUS CYCLE

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SUMMARY.

Two groups, each of 10 Jersey cows, were observed for periods of 99 and 86 days, respectively, in succeeding years.

Mean body temperature was 100.2±0.8°F (n=1,824) in the morning and 101.4±0.8°F. (n=1,764) in the evening. Eighteen oestrous periods were detected in the morning; 13 were noted in the evening; and in 6 cases oestrus was evident at morning and evening.

No correlation could be established between stages of the oestrous cycle and morning or evening temperature trends, except that a thermogenic response ($P<0.001$) in the presence of oestrus was noted.

Preliminary investigations indicate that the response persists for the duration of oestrus. It is suggested that the use of the temperature rise at oestrus could be an adjunct to more conventional methods of oestrus detection if exercised with care and judgment.

The significance of the thermogenic response at oestrus in relation to metabolic processes is considered, and attention is drawn to its possible importance in heat regulation and in adaptation to unfavourable environments.

I. INTRODUCTION.

The psychic changes associated with maturation of the Graafian follicle in the cow are normally of sufficient intensity as to allow the competent observer to define the presence of oestrus. On the other hand, the determination of ovulation requires intricate techniques, but is facilitated by the readiness with which the cow's ovaries may be examined by rectal palpation.

However, under various circumstances the intensity and duration of oestrous symptoms may be partially or largely suppressed. In the application of artificial insemination to beef cattle under Queensland conditions "teasers" such as vasectomised bulls have been used to identify cows in oestrus, though the efficiency of this technique has been called into doubt by Wiltbank, Burris and Priode (1956) and by J. F. Kennedy in a private communication, and such bulls may provide a reservoir for venereal pathogens (J. N. Shelton and L. Donaldson, personal communication).

Conventional methods of detecting oestrus and ovulation have presented grave difficulties and sources of error in the study of pathological conditions such as anoestrus. Furthermore, the lack of more objective means of defining these phenomena constitutes a problem in the study of endocrine therapy and related fields.

From time to time various techniques have been proposed as adjuncts to the subjective determination of oestrus. For example, some success has been reported in the correlation of cervical mucus crystallisation with the oestrous cycle in beef cattle (Alliston, Patterson and Ulberg 1958). However, anomalies with this method have been noted in the anoestrous state (Fallon and Crofts 1959).

Variations in body temperature throughout the menstrual cycle were reported by Van de Velde (1904) and have since been used extensively for the determination of ovulation in women (Zuck 1938; Viergiver and Pommerenke 1946). The technique has been extended to other species and a biphasic temperature curve during the cow's oestrous cycle has been reported (Vollman and Vollman 1942).

The widespread nature of anoestrus in south-eastern Queensland (McTackett 1956) and a high incidence of atypical oestrous cycles (Fallon 1958) have been reported. Ovulation patterns in such conditions have not been thoroughly investigated because of the practical difficulties in arranging extensive programmes of ovarian examinations.

The present study was undertaken to determine if temperature observations made in conjunction with normal milking-shed routine might be of sufficient accuracy to reflect ovarian activity.

II. MATERIAL AND METHODS.

The observations were made in a commercial herd of Jersey cows at Hunchy (26°29′S, 152°80′E) near Nambour in south-eastern Queensland. Two observation periods were used—from Nov. 18, 1957, to Feb. 24, 1958, and from Sept. 17 to Dec. 11, 1958. During each period the rectal temperatures of 10 cows was taken twice daily at milking times (approximately 6.30 a.m. and 6.00 p.m.) with clinical thermometers graduated at intervals of 0·2 deg. F. Determination of oestrus was based on behavioural changes in the trial animals.

III. RESULTS.

Calving and oestrus data are presented in Table 1. Considerable incidence of anoestrus and atypical oestrous cycles is evident in the records. In the present study the temperature observations have been assigned in each case to the nearest observed oestrus. Temperature records taken more than 10 days before or after an observed oestrus are not considered here, except to say that the mean rectal temperature for the total of 1,824 morning records was $100{\cdot}2 \pm 0{\cdot}8$ deg. F. and for 1,764 evening records $101{\cdot}4 \pm 0{\cdot}8$ deg. F.

Table 1.

Calving and Oestrus Data.

Cow.	Calving Date.	Oestrus Dates.
A ..	20/6/57	14/9, 6/10, 19/1 (x), 4/2 (x)
B ..	1/9/57	22/11 (x)
C ..	30/9/57	16/2 (x)
D ..	29/8/57	29/1 (x), 25/2 (x), 4/3 (x)
E ..	29/8/57	19/10, 7/11, 26/11 (x)
F ..	2/10/57	26/2, 5/3 (x)
G ..	6/9/57	15/10, 5/11, 26/11 (x)
H ..	1/9/57	26/10, 3/11, 22/11 (x), 12/12 (x)
I ..	1/9/57	26/11 (x), 3/12 (x)
J ..	27/9/57	25/11 (x), 16/12 (x), 5/2 (x), 28/2 (x)
K ..	21/8/58	30/9, 21/10, 13/11, 4–5/12 (x)
L ..	13/8/58	5/9, 27/9, 19/10, 10–11/11, 30/11 (x)
M ..	26/8/58	27/10, 13–14/11, 1/12 (x)
N ..	24/8/58	21/12 (x)
O ..	4/9/58	5/12 (x), 26/12 (x), 15/1 (x)
P ..	11/9/58	23/11 (x), 30/11–1/12 (x)
Q ..	3/9/58	20/10, 28/10, 17/11 (x)
R ..	6/9/58	10/10, 8/11, 16/11 (x)
S ..	6/9/58	10/11, 17/11 (x)
T ..	11/9/58	23/10, 22/11 (x)

"x" denotes service, natural or artificial.

Available temperature data for each day of the oestrous cycle have been bulked and averaged. The calculated means are set out in Table 2: 18 oestrous periods were detected in the morning, 13 were noted in the evening, and in 6 cases oestrus was evident at morning and evening milkings. Body temperature trends throughout the oestrous cycle are illustrated in Fig. 1.

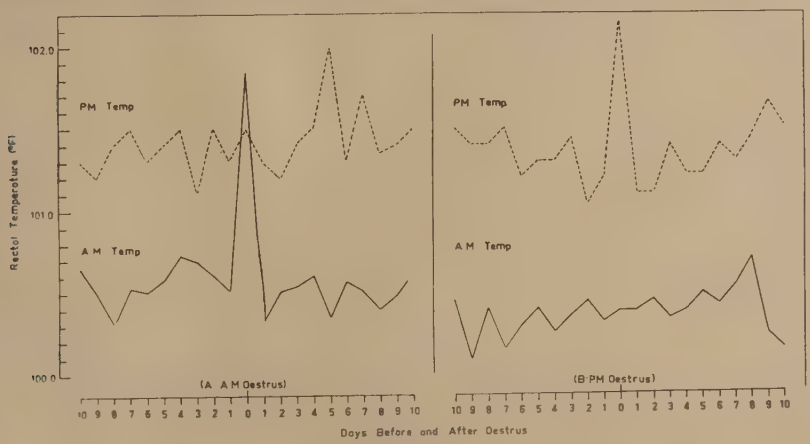

Fig. 1.

Variations in Body Temperature in Relation to the Oestrous Cycle.

No correlation between body temperature and the stage of the cycle could be established, though a thermogenic response in the presence of oestrus was highly significant ($P<0{\cdot}001$). The distribution of body temperature on the day of oestrus in relation to the specific time of oestrus is presented in Fig. 2.

Table 2.

Body Temperature and the Oestrous Cycle.

Days Before and After Oestrus.	Morning Oestrus.				Evening Oestrus.			
	n	A.M. Temp.	n	P.M. Temp.	n	A.M. Temp.	n	P.M. Temp.
10	14	100·64	11	101·32	8	100·45	10	101·50
9	14	100·50	11	101·20	9	100·09	11	101·43
8	18	100·32	11	101·42	9	100·38	13	101·43
7	19	100·53	15	101·48	10	100·16	14	101·47
6	19	100·51	15	101·28	10	100·28	14	101·23
5	19	100·58	15	101·44	10	100·42	15	101·29
4	23	100·73	17	101·52	12	100·27	17	101·29
3	24	100·69	17	101·14	11	100·36	17	101·44
2	24	100·61	15	101·49	11	100·44	18	101·06
1	24	100·52	16	101·34	12	100·32	18	101·19
0	24	101·85	16	101·53	12	100·38	19	102·16
1	24	100·33	16	101·28	12	100·38	18	101·10
2	24	100·51	17	101·18	12	100·45	18	101·08
3	24	100·54	16	101·44	12	100·33	19	101·37
4	22	100·60	15	101·53	12	100·38	16	101·23
5	21	100·35	13	101·97	11	100·49	15	101·23
6	21	100·57	10	101·30	11	100·42	16	101·38
7	19	100·51	12	101·67	11	100·53	17	101·27
8	19	100·40	11	101·35	11	100·71	16	101·46
9	18	100·48	11	101·40	10	100·24	14	101·66
10	17	100·62	10	101·54	10	100·14	14	101·50
Mean (Oestrus excepted)	407	100·53	274	101·42	214	100·38	310	101·32

(N.B.—Rectal temperatures were recorded in degrees Fahrenheit).

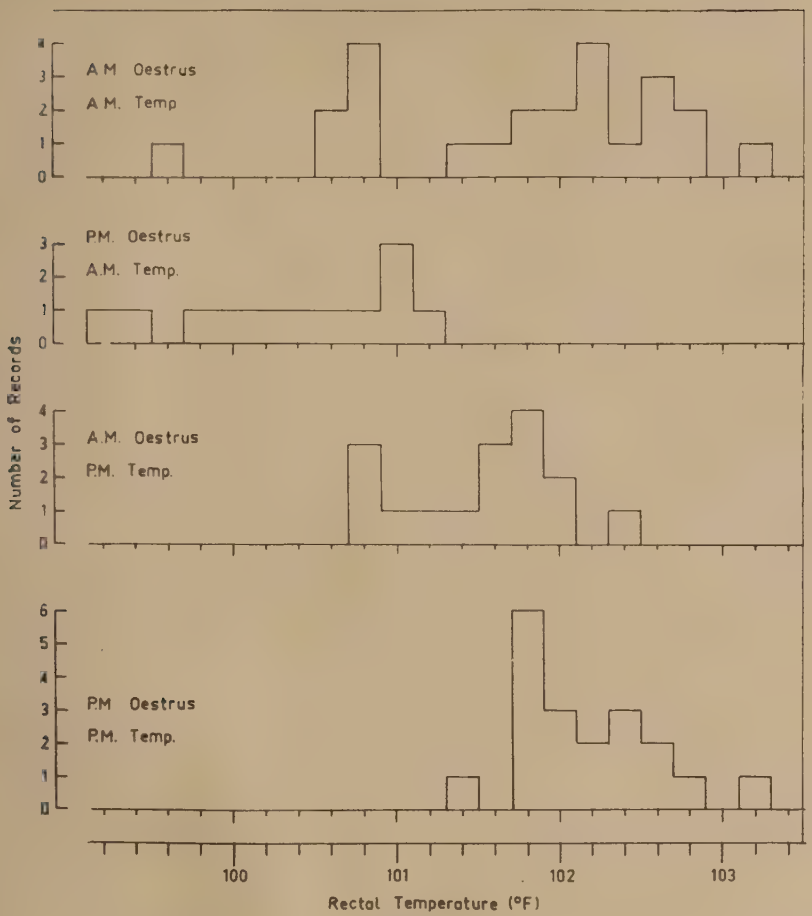

Fig. 2.

Distribution of Body Temperature Records on the Day of Oestrus in Relation to the Time of Oestrus Observation.

IV. DISCUSSION.

Since the present study was commenced, a similar investigation has been reported from Beltsville (Wrenn, Bitman and Sykes 1958), where vaginal temperatures of cows were recorded between 11 a.m. and noon. The workers were able to define a biphasic curve in body temperature in association with about 90 per cent. of 54 oestrous cycles, and they attributed the pattern to the thermogenic influence of endogenous progesterone.

In the present study, however, it was found that except for temperatures recorded during oestrus the daily variation was random in nature throughout

the cycle, with a standard deviation of about 0·6 deg. F. Similarly, Greenstein, Murray and Foley (1958) found daily temperature records unreliable indicators of individual cyclic behaviour patterns. In addition, they were unable to demonstrate a thermogenic response to exogenous progesterone in intact heifers.

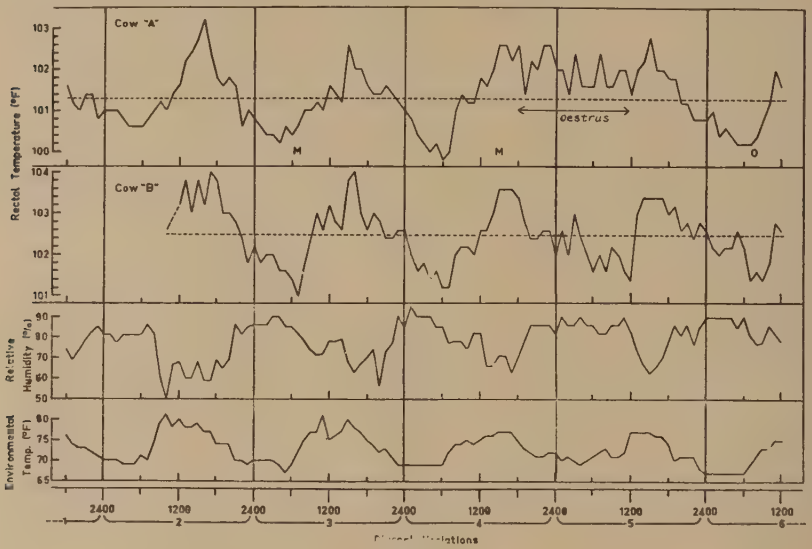

Fig. 3.

Body Temperature Trends in Terminal Stages of Oestrous Cycle in Cow A, Compared with Pregnant Cow B. (M: cervical mucus discharge; O: ovulation).

It should be noted that such animal studies as are reported here differ from the human investigations in that in the latter work temperatures are measured while the subject is in the "post-absorptive state."

The significant temperature response at oestrus is of particular interest. The phenomenon was not reported by Swiss workers (Vollman and Vollman 1942), but was noted by Wrenn, Bitman and Sykes (1958). Present data indicate that the temperature elevation is of such short duration that it could have been missed in a number of cases if recordings had been made only once daily.

Preliminary studies of the nature of the thermogenic response at oestrus have been initiated. In one investigation (see Fig. 3) the experimental animal was an aged cow afflicted with a long-standing dislocation of the hip joint. Thus, the activity response which is characteristic of oestrus (Wang 1923; Altmann 1941; Farris 1954) was markedly reduced. However, the thermogenic response persisted for the duration of oestrus. Normal diurnal body temperature rhythm was resumed before ovulation occurred.

While the temperature response at oestrus has been demonstrated, it is apparent that the use of this fact as an adjunct to more conventional methods of oestrus detection would need to be exercised with care and judgment. The significance of the individuality of the cow in respect of repeatability of body temperature was discussed by Kriss (1921). Such individuality in behaviour may explain the failure of some workers (e.g., Melampy, Hanka and Enss 1956) to demonstrate a thermogenic response at oestrus.

It remains for the nature of the temperature response in the oestrous cow to be elucidated. While augmented muscular activity undoubtedly contributes to the increased heat production, small-animal studies (Lee and Van Buskirk 1928) suggest that basal metabolism may be increased at or about oestrus independent of activity trends.

Soliman and Reineke (1954) demonstrated increased thyroid activity during oestrus in the rat, and more recently experimental evidence has been presented indicating that the increased elaboration of oestrogens in late pregnancy in the cow may exert a thyrotrophic effect (Pipes, Premachandra and Turner 1958). It is possible that such an effect might operate during oestrus in the cow, but apparently the thyroid status of the oestrous cow has not been critically examined. It is relevant that Spielman, Petersen, Fitch and Pomeroy (1945) demonstrated a reversible suppression of oestrus in the thyroidectomised heifer, although ovulation was apparently not impaired.

It is considered that the thermogenic response of the cow at oestrus is of more than academic interest in relation to bovine reproduction in tropical regions. Low calving rates are a feature in such areas (Hart and Guilbert 1928; Chester 1952; Van Rensburg 1956; Beattie 1956). This may be explained in part by the tendency for cattle in unfavourable environments to pass into anoestrus in alternate years (Hart 1955). While the condition has a nutritional basis (Guilbert and Hart 1946), its etiology is complex and may be appropriately considered in terms of the "adaptive sterility" hypothesis of Schaetz (1956).

Although the role of temperature in the suppression of oestrus in cattle is not clear (Hammond 1955), it is apparent that the thermogenic component of oestrus offers an interesting field of consideration in respect of heat-regulating mechanisms and adaptation of cattle to unfavourable environments.

V. ACKNOWLEDGEMENTS.

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INDEX TO VOL. 16

A

PAGE

"A Biochemical Study of 141 Strains of Enterobacteriaceae Isolated from Tissues and Fluids of Animal Origin" 419

"A Crown Rot of Strawberry Caused by *Phytophthora parasitica*" 73

"A Note on Foliar Diagnosis of Boron Deficiency in Beetroot" 85

"A Preliminary Investigation into Control of False Wireworm on the Darling Downs" 251

"A Study of Three Important Pasture Mixtures in the Queensland Subtropics: Progress Report, 1955–1958" 199

Agnew, G. W. J.—

"French Bean Variety and Spacing Trial" 283

Apple storage 291

B

"Bag Treatment to Control Pests of Hooded Sorghum" 245

Banana, effect of growth regulators 87

Beames, R. M.—

"Molasses and Urea as a Supplement to Low Quality Pasture Hay for Cattle" 223

"Note on the Effect of Molasses on Liver Copper Concentrations in Cattle" 233

Beetroot, boron deficiency 85

Bengston, M. (with A. W. S. May)—

"Studies on Codling Moth Control in the Granite Belt" 355

Blake, J. R. (with C. D. Stevenson)—

"Effects of Growth Regulating Substances on the Ripening of Detached Banana Fruits" 87

"Blue Mould (*Peronospora tabacina* Adam) of Tobacco in North Queensland: Some Aspects of Chemical Control" 299

Boron studies 79, 85

"Boron Studies on a Krasnozem Soil in Queensland" 79

Bowen, G. D.—

"Field Studies on Nodulation and Growth of *Centrosema pubescens* Benth." 253

"Specificity and Nitrogen Fixation in the Rhizobium Symbiosis of *Centrosema pubescens* Benth." 267

Bowen, G. D. (with Margaret M. Kennedy)—

"Effect of High Soil Temperatures on *Rhizobium* spp. 177

Brimblecombe, A. R.—

"Studies of the Coccoidea. 8. Three New Genera and Sixteen New Species of Aspidiotini" 121

"Studies of the Coccoidea. 9. New Species of *Apiomorpha* from Queensland" 157

"Studies of the Coccoidea. 10. New Species of Diaspididae" 381

PAGE

Burns, M. A. (with N. D. Young and N. F. Fox)—

"A Study of Three Important Pasture Mixtures in the Queensland Subtropics: Progress Report, 1955–1958 199

Butter, copper content 49

Butterfat—

Carotene and vitamin A contents 1

Iodine value and softening point and churning temperature 31

C

Cannon, R. C. (with E. T. Prodonoff)—

"Trials with CMU Weedicides in Queensland Pineapple Plantations" .. 217

Cattle—

feed supplements 223

oestrus studies 431, 439

Centrosema pubescens—

nodulation and growth 253

specificity and nitrogen fixation 267

Coccoidea studies 121, 157

Codling moth control 355

Colbran, R. C.—

"Strawberry Root-knot Nematode Investigations in Queensland" 365

Copper—

content of butters 49

deficiency in sheep 93

in liver of cattle 233

Cotton, Heliothis pest 165

Crofts, J. M. (with G. R. Fallon)—

"Some Aspects of Oestrus in Cattle, with Reference to Fertility on Artificial Insemination. 2. Crystallisation Patterns in Cervico-vaginal Mucus" .. 431

D

"DDT for the Protection of Stored Potatoes" 329

E

"Effect of High Soil Temperatures on *Rhizobium* spp." 177

"Effect of Maturity and Tree Age on the Behaviour of Queensland-grown Delicious Apples Stored at 34–36°F." 291

"Effects of Growth Regulating Substances on the Ripening of Detached Banana Fruits" 87

Enterobacteriaceae isolations 419

F

PAGE

Fallon, G. R.—
"Some Aspects of Oestrus in Cattle, with Reference to Fertility on Artificial Insemination. 3. Body Temperature and the Oestrous Cycle" 439

Fallon, G. R. (with J. M. Crofts)—
"Some Aspects of Oestrus in Cattle, with Reference to Fertility on Artificial Insemination. 2. Crystallisation Patterns in Cervico-vaginal Mucus" .. 431

Ferricks, M. B.—
"The Copper Content of Some Queensland Butters" 49

"Field Studies on Nodulation and Growth of *Centrosema pubescens* Benth." .. 253

Fox, N. F. (with N. D. Young and M. A. Burns)—
"A Study of Three Important Pasture Mixtures in the Queensland Subtropics: Progress Report, 1955–1958" 199

"French Bean Variety and Spacing Trial" 283

"Freeze-drying of Starter Cultures" 409

G

Gartner, R. J. W.—
"Variations in the Carotene and Vitamin A Contents of Queensland Butterfats" 1

H

Harvey, J. M. (with G. R. Moule and A. K. Sutherland)—
"Occurrence and Correction of Copper Deficiency of Sheep in North-western Queensland" 93

"Heliothis as a Pest of Cotton in Central Queensland" 165

Hooper, G. H. S.—
"A Preliminary Investigation into Control of False Wireworm on the Darling Downs" 251

Hughes, J. D.—
"A Note on Foliar Diagnosis of Boron Deficiency in Beetroot" 85

"Boron Studies on a Krasnozem Soil in Queensland" 79

I

"Iodine Value and Softening Point and Churning Temperature of Some Queensland Butterfats" 31

K

Kennedy, Margaret M. (with G. D. Bowen)—
"Effect of High Soil Temperatures on *Rhizobium* spp." 177

M

PAGE

May, A. W. S.—
" DDT for the Protection of Stored Potatoes " 329

May, A. W. S. (with M. Bengston)—
" Studies on Codling Moth Control in the Granite Belt " 355

McCarthy, G. J. P. (with D. S. Teakle)—
"A Crown Rot of Strawberry Caused by *Phytophthora parasitica* " 73

" Mild Strain Protection as a Means of Reducing Losses from the Queensland Woodiness Virus in the Passion Vine " 371

Mitchell, W. D.—
" Iodine Value and Softening Point and Churning Temperature of Some Queensland Butterfats " 31

Molasses—
effect on liver copper 233
supplement to hay 223

" Molasses and Urea as a Supplement to Low Quality Pasture Hay for Cattle " 223

Moule G. R. (with A. K. Sutherland and J. M. Harvey)—
" Occurrence and Correction of Copper Deficiency of Sheep in North-western Queensland " 93

N

Nematode, strawberry 365

" Note on the Effect of Molasses on Liver Copper Concentrations in Cattle " .. 233

O

" Occurrence and Correction of Copper Deficiency of Sheep in North-western Queensland " 93

P

Passion vine woodiness 371

Passlow, T.—
" Heliothis as a Pest of Cotton in Central Queensland " 165
" Bag Treatment to Control Pests of Hooded Sorghum " 245

Pasture—
grazing studies 199
white grub control 239

Phytophthora parasitica on strawberry 73

Pineapples, weedicides 217

Pont, W.—
" Blue Mould (*Peronospora tabacina* Adam) of Tobacco in North Queensland : Some Aspects of Chemical Control " 299

PAGE

Potato storage with DDT 329

Prodonoff, E. T. (with R. C. Cannon)—

"Trials with CMU Weedicides in Queensland Pineapple Plantations" 217

"*Puccinia argophylli* sp. n. on *Argophyllum nullumense* in Queensland" 91

R

"Removal of Lead Arsenate From Green Tobacco Leaf" 249

Rhizobium—

Centrosema pubescens 253

effect of soil temperature 177

Rossiter, P. D. (with R. J. Talbot)—

"Soil Survey of Coolum Field Station, South-eastern Queensland" 53

S

Saunders, G. W.—

"Toxicities of Insecticides to Pasture White Grubs in Queensland" 239

Schubert, W. F.—

"Freeze-drying of Starter Cultures" 409

Sheep, copper deficiency 93

Simmonds, J. H.—

"Mild Strain Protection as a Means of Reducing Losses From the Queensland Woodiness Virus in the Passion Vine" 371

Smith, W. A.—

"Removal of Lead Arsenate from Green Tobacco Leaf" 249

Soil—

boron content 79

survey, Coolum Field Station 53

"Soil Survey of Coolum Field Station, South-eastern Queensland" 53

"Some Aspects of Oestrus in Cattle, with Reference to Fertility on Artificial Insemination. 2. Crystallisation Patterns in Cervico-vaginal Mucus" 431

"Some Aspects of Oestrus in Cattle, with Reference to Fertility on Artificial Insemination. 3. Body Temperature and the Oestrous Cycle" 439

Sorghum, control of pests 245

"Specificity and Nitrogen Fixation in the Rhizobium Symbiosis of *Centrosema pubescens* Benth." 267

Starter cultures, freeze-drying 409

Stevenson, C. D.—

"Effect of Maturity and Tree Age on the Behaviour of Queensland-grown Delicious Apples Stored at 34–36°F." 291

Stevenson, C. D. (with J. R. Blake)—

"Effects of Growth Regulating Substances on the Ripening of Detached Banana Fruits" 87

PAGE

Strawberry—
crown rot 73
root-knot nematode 73

"Strawberry Root-knot Nematode Investigations in Queensland" 365

"Studies of the Coccoidea. 8. Three New Genera and Sixteen New Species of Aspidiotini" 121

"Studies of the Coccoidea. 9. New Species of *Apiomorpha* from Queensland" .. 157

"Studies of the Coccoidea. 10. New Species of Diaspididae" 381

"Studies on Codling Moth Control in the Granite Belt" 355

Sutherland, A. K. (with G. R. Moule and J. M. Harvey)—
"Occurrence and Correction of Copper Deficiency of Sheep in North-western Queensland" 93

T

Talbot, R. J. (with P. D. Rossiter)—
"Soil Survey of Coolum Field Station, South-eastern Queensland" 53

Teakle, D. S. (with G. J. P. McCarthy)—
"A Crown Rot of Strawberry Caused by *Phytophthora parasitica*" 73
"*Puccinia argophylli* sp. n. on *Argophyllum nullumense* in Queensland" .. 91

"The Copper Content of Some Queensland Butters" 49

Tobacco—
blue mould control 299
residues 249

"Toxicities of Insecticides to Pasture White Grubs in Queensland" 239

"Trials with CMU Weedicides in Queensland Pineapple Plantations" 217

Turner, Elizabeth—
"A Biochemical Study of 141 Strains of Enterobacteriaceae Isolated from Tissues and Fluids of Animal Origin" 419

U

Urea as hay supplement 223

V

"Variations in the Carotene and Vitamin A Contents of Queensland Butterfats" 1

W

Weedicides in pineapples 217

Wireworm control 251

Y

Young, N. D. (with N. F. Fox and M. A. Burns)—
"A Study of Three Important Pasture Mixtures in the Queensland Subtropics: Progress Report, 1955–1958" 199

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